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085895

Filed 1800

PATENT APPLICATION



08585895

Date Entered or Counted

CONTENTS *Hqj*

APPROVED FOR LICENSE

INITIALS

Date Received or Mailed

9/1/97

1. Application *24 Sheets papers.*
2. *Raw Request Hearing (OK)*
3. *Ex. L. Declaration*
4. *Petition (S3)*
5. *Petition granted (S3)*
6. *Best. 30 days*
7. *Pre Amdt / A*
8. *I.O.S. w/ Attach*
9. *I.D.S.*
10. PETITION TO EXPEDITE (PETITION)
11. *I.D.S.*
12. *I.D.S.*
13. *I.D.S.*
14. *Ex. L. Declaration*
15. *Ex. L. Declaration / Amdt / A*
16. *INTO DISCL Amdt*
17. *REI 3 mos*
18. *Ext. of Time (3 months)*
19. *Amdt C / Declaration / CCF Disk*
20. *Power of Attorney*
21. *Raw Request Hearing*
22. *Ex. 3 mos*
23. *Declaration*
24. *Ext. of Time 1 month*
25. *Dec. of*
26. *Amdt - D*
27. *Letter of Suspension*
28. *SID*
29. *S.I.D.S.*
30. *Ref. 3 months*
31. *Power of Attorney*
32. *Interview Summary*

8/1/96
APR 14 1996
5/24/96
7/22/96
11/25/96
8-14-96
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12-23-96
06-25-96
02-11-97
03-24-97
1/23/97
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7/23/98
7-27-98
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Oct. 28, 1999
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10-22-00
6-28-00

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|---------------------------------|--|----------------------------|--|--------------------------|--|
| Class 435 | | Subclass 69.4 | | ISSUE CLASSIFICATION | |
| UTILITY SERIAL NUMBER 585895 | | PATENT DATE JUN 12 2001 | | PATENT NUMBER 6245530 | |
| SERIAL NUMBER 08/585,895 | | FILING DATE 01/12/96 | | CLASS 435 | |
| SUBCLASS 69.4 | | GROUP ART UNIT 124 | | EXAMINER 52211 | |

APPLICANTS: KARI ALITALO, ESPOO, FINLAND; V. ALITALO, FINLAND

CONTINUING DATA***
 VERIFIED THIS APPLN IS A CIP OF 02/340,011 11/14/94
 BXL
 **FOREIGN/PCT APPLICATE NOT VERIFIED
 NONE
 BXL

| | | | | | | | |
|---|---|-----------------------------|---------------------|--------------------|--------------------|----------------------------|--------------------------------|
| Foreign priority claimed 35 USC 119 conditions met <input type="checkbox"/> yes <input checked="" type="checkbox"/> no | AS FILED <input checked="" type="checkbox"/> | STATE OR COUNTRY FINLAND | SHEETS DRWGS. 30 | TOTAL CLAIMS 35 | INDEP. CLAIMS 1 | FILING FEE RECEIVED 124 | ATTORNEY'S DOCKET NO. 52211 |
| Verified and Acknowledged Examiner's Initials MARSHALL OTTOLE GERSTEIN MURPHY 6300 SEARS TOWER 1233 SOUTH WACKER DRIVE CHICAGO IL 60606-8402 | | | | | | | |

RECEPTOR LEGEND

U.S. DEPT. OF COMM./PAT. & TM—PTO-436L (Rev.12-94)

| | |
|---|---|
| 04/05/01 Formal Drawings (30 shts) set L 01/24/01 | |
| PARTS OF APPLICATION FILED SEPARATELY | |
| NOTICE OF ALLOWANCE MAILED 19-24-00 | CLAIMS ALLOWED Total Claims 35 Print Claim 1 |
| ISSUE FEE (W) Amount Due 1240 Date Paid 1-20-91 | DRAWING Sheets Drwg. 2430 Figs. Drwg. 2631 Print Fig. None |
| Label Area | CHRISTINE SAUND PATENT EXAMINER Christine Saund Primary Examiner PREPARED FOR ISSUE |
| WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only. | |

Form PTO-436L (Rev. 8/92)

Formal Drawings (30 shts) set L

BXL

| POSITION | ID NO. | DATE |
|-------------|--------|---------|
| CLASSIFIER | | |
| EXAMINER | 4/6 | 2-22-96 |
| TYPIST | | 4/15/96 |
| VERIFIER | | |
| CORPS CORR. | | |
| SPEC. HAND | 509 | 7-1-96 |
| FILE MAINT. | 323 | 5-14 |
| DRAFTING | | |

INDEX OF CLAIMS

| Claim | Final | Original | Date |
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SYMBOLS
✓ Rejected
- Allowed
(Through number) Canceled
N Restricted
I Non-elected
A Interference
O Appeal
O Rejected

| Claim | Final | Original | Date |
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| | | Date received (Incl. C. of M.) or Date Mailed | Date received (Incl. C. of M.) or Date Mailed |
|------------------------------|---------------------|--|--|
| 1. Application _____ papers. | | | |
| 2 | | | |
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| 33 | Effect time Warrant | Aug. 10, 2000 | |
| 34 | Declaration | Aug. 10, 2000 | |
| 35 | Amend E | Aug. 10, 2000 | |
| 36 | Declaration | 8/14/00 | |
| 37 | Amend of Motion | 10-24-00 1917 | |
| 38 | Amend G | 01/24/01 | |
| 39 | PTOL 271 | 02/24/01 | |
| 40 | Drawings | 1/24/01 | |
| 41 | | | |
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00 58589

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:)
Alitalo et al.)
Serial No.: Not yet assigned)
Filed: Herewith)
For: Receptor Ligand)
Group Art Unit: Not yet assigned)
Examiner: Not yet assigned)

) "EXPRESS MAIL"
) Mailing label No. EG473137204US
) Date of Deposit: January 12, 1996
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) 37 CFR §1.10 on the date indicated above
) and is addressed to the Assistant
) Commissioner for Patents,
) Washington, D.C., 20231.
) David A. Gass
) David A. Gass

STATEMENT PURSUANT TO 37 C.F.R. §1.821(f)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer readable forms of the sequence listing that is part of the above-identified application and that are filed herewith are the same.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Dated: January 12, 1996

David A. Gass
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 1995

Application or Docket Number

08/585895

CLAIMS AS FILED - PART I

| FOR | (Column 1) NUMBER FILED | (Column 2) NUMBER EXTRA |
|----------------------------------|----------------------------|----------------------------|
| BASIC FEE | | |
| TOTAL CLAIMS | 16 minus 20 = | * |
| INDEPENDENT CLAIMS | 3 minus 3 = | * |
| MULTIPLE DEPENDENT CLAIM PRESENT | | |

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY

| RATE | FEE |
|--------|--------|
| | 375.00 |
| x\$11= | |
| x39= | |
| +125= | |
| TOTAL | |

OTHER THAN SMALL ENTITY

| RATE | FEE |
|--------|--------|
| | 750.00 |
| x\$22= | |
| x78= | |
| +250= | |
| TOTAL | 750 |

CLAIMS AS AMENDED - PART II

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA |
|--|--|--|-----------------------------|
| Total | 23 Minus | 20 | = 13 |
| Independent | 3 Minus | 3 | = 0 |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM | | | |

SMALL ENTITY

| RATE | ADDITIONAL FEE |
|------------------|---------------------|
| x\$11= | \$143 ⁰⁰ |
| x39= | \$82 ⁰⁰ |
| +125= | \$135 ⁰⁰ |
| TOTAL ADDIT. FEE | \$360 ⁰⁰ |

OTHER THAN SMALL ENTITY

| RATE | ADDITIONAL FEE |
|------------------|----------------|
| x\$22= | |
| x78= | |
| +250= | |
| TOTAL ADDIT. FEE | |

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA |
|--|--|--|-----------------------------|
| Total | 0 Minus | 37 | = |
| Independent | 0 Minus | 5 | = |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM | | | |

| RATE | ADDITIONAL FEE |
|------------------|----------------|
| x\$11= | |
| x39= | |
| +125= | |
| TOTAL ADDIT. FEE | |

| RATE | ADDITIONAL FEE |
|------------------|----------------|
| x\$22= | |
| x78= | |
| +250= | |
| TOTAL ADDIT. FEE | |

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA |
|--|--|--|-----------------------------|
| Total | 35 Minus | 37 | = |
| Independent | 18 Minus | 5 | = 2 |
| FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM | | | |

| RATE | ADDITIONAL FEE |
|------------------|----------------|
| x\$11= | |
| x39= | 78 |
| +125= | C |
| TOTAL ADDIT. FEE | |

| RATE | ADDITIONAL FEE |
|------------------|----------------|
| x\$22= | |
| x78= | |
| +250= | |
| TOTAL ADDIT. FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" in THIS SPACE is less than 20, enter "20."
 *** If the "Highest Number Previously Paid For" in THIS SPACE is less than 3, enter "3."
 The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.



US006245530B1

(12) **United States Patent**
Alitalo et al.

(10) Patent No.: **US 6,245,530 B1**
(45) Date of Patent: **Jun. 12, 2001**

(54) **RECEPTOR LIGAND**

(75) Inventors: Kari Alitalo, Espoo (FI); Vladimir
Joukov, Boston, MA (US)

(73) Assignees: Ludwig Institute for Cancer
Research, New York, NY (US);
Helsinki University Licensing, Ltd.
OY, Helsinki (FI)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: 08/585,895

(22) Filed: Jan. 12, 1996

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/510,133, filed on
Aug. 1, 1995.

(51) Int. CL⁷ C12N 15/12; C12N 15/63;
C12N 5/10; C12N 5/16

(52) U.S. Cl. 435/69.4; 435/70.1; 435/325;
435/320.1; 536/23.51; 530/399; 935/13

(58) Field of Search 536/23.51; 435/252.3;
435/254.11; 320.1; 419; 69.4; 70.1; 325;
530/399; 935/13

(56) **References Cited****U.S. PATENT DOCUMENTS**

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|-----------|--------|---------------------|-----------|
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| 5,932,540 | 8/1999 | Jing-Shan Hu et al. | |
| 5,935,820 | 8/1999 | Jing-Shan Hu et al. | |

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| | | |
|--------------|---------|------|
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| WO 95/24473 | | |
| A1 | 9/1995 | (WO) |
| WO 95/33050 | 12/1995 | (WO) |
| WO 95/33772 | 12/1995 | (WO) |
| WO 96/11269 | | |
| A2 | 4/1996 | (WO) |
| WO 96/30046 | 10/1996 | (WO) |
| WO 96/39421 | 12/1996 | (WO) |
| WO 96/39515 | 12/1996 | (WO) |
| 97/05250 | 2/1997 | (WO) |
| 97/09427 | 3/1997 | (WO) |
| 97/17442 | 5/1997 | (WO) |

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5' EST-STS Accession No. H05177, Jun. 21, 1995.*
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5' EST-STS Accession No. H07991, Jun. 23, 1995.*
Hillier et al. yd29f07.r1 *Homo sapiens* cDNA clone 109669
5' similar to SP:BAR3_CHITE Q03376 Balbiani Ring
Protein 3. EST-STS Accession No. T81690, Mar. 15, 1995.*
Auffray et al. *H. sapiens* partial cDNA sequence: clone
c-1wf11. EST-STS Accession No. Z44272, Nov. 6, 1994.*

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Provisional application No. 60/003,491, James Lee and William Wood, Sep. 8, 1995.

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Achen, M.G. et al., "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)." *Proceedings of the National Academy of Science, USA*, 95:548-553 (Jan., 1998).

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(List continued on next page.)

Primary Examiner—Christine Saoud

(74) Attorney, Agent, or Firm—Marshall, O'Toole, Gerstein, Murray & Borun

(57) **ABSTRACT**

Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligands, pharmaceutical compositions and diagnostic reagents.

35 Claims, 30 Drawing Sheets

SEARCHED

| Class | Sub | Date | Exmr. |
|-------|--------|----------|-------|
| 556 | 2351 | 5/25/97 | ENL |
| 555 | 2353 | | |
| | 357/11 | | |
| | 2012 | | |
| | 779 | | |
| 1005 | 1694 | 3/19/98 | ED |
| 1005 | 1694 | 3/28/00 | CL |
| 435 | 1694 | 10/20/00 | CL |
| | 701 | | |
| | 325 | | |
| | 6201 | | |
| 536 | 2351 | | |
| 530 | 399 | | |
| 985 | 13 | | |

SEARCH NOTES

| | Date | Exmr. |
|--|----------|-------|
| SEQ ID NOS 32 and 33, excerpts attached. | 11/15/96 | ENL |
| USPAT, MEDLING, WPIOS searched - see attached. | 5/25/97 | ENL |
| Update - see search notes in file | 3/19/98 | KPS |
| seq. search | 2/18/00 | CL |
| SEQ ID NO: 32-33 | 3/28/00 | CL |
| update | 10/20/00 | CL |
| update | | |

INTERFERENCE SEARCHED

| Class | Sub | Date | Exmr. |
|-------|------|----------|-------|
| 551 | 1694 | 10/20/00 | CL |
| | 576 | | |
| | 325 | | |
| | 6201 | | |
| | 2351 | | |
| | 399 | | |
| | 13 | | |

Jul. 23. 1998 5:15PM MARSHALL, OTOOLE

No. 8729 P. 2/4

From: 0819

#23

Patent Clerk
PATENT

28967/33072

7/24/98

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage
) prepaid, in an envelope addressed to:
) Assistant Commissioner for Patents
) Washington, D.C. 20231, on this date:

) Dated: Nov. 26, 1997

) *David A. Gass*

) David A. Gass

) Registration No. 38,153

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.

2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Kari Alitalo
Kari Alitalo



American Type Culture Collection

12341 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 231-5520 Telex: 816-455 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki
Attention: Kari Alitalo
Molecular/Cancer Biology Laboratory
P.O. Box 21 (Haartmaninkatu 3)
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor: ATCC Designation

Plasmid, FLT4-L 97231

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Annette L. Bade, Director, Patent Depository

Date: August 9, 1995

cc: Thomas C. Meyers

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PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Alitalo *et al.*

Serial No. 08/585,895

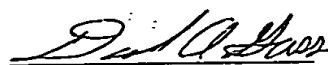
Filed: January 12, 1996

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Art Unit: 1646

Examiner: Saoud

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) date: July 23, 1998

) 
) David A. Gass
) Attorney for Applicants
)

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. § 1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In an official action mailed March 24, 1998, the U.S. Patent and Trademark Office (the Patent Office) allowed claims 33-36, but rejected claims 1, 3-5, 7, 11, 18-32, and 37-38 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Patent Office also objected to an amendment under § 132, alleging that the amendment introduced new matter. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment has been timely filed with a petition and fee for one month extension of time, extending the shortened statutory period to July 24, 1998.

AMENDMENTS

In the specification:

Please amend the specification as set forth below:

Please delete the amendment to the priority claim at page 1, line 3, filed on August 12, 1996, and substitute therefor the following updated priority claim: ~~This application~~^{AND} is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755.

At page 5, line 21, delete "SEQ ID NO: 2" and substitute therefor --SEQ ID NO: 33--.

At page 5, line 31, delete "polypeptide" and substitute therefor --polypeptides--.

Please cancel the amendment to page 29, line 1, of the specification made on November 26, 1996, and substitute therefor the following amendment at the same location: --The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a nucleotide sequence that includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The nucleotide sequence set forth in SEQ ID NO: 44 encodes the 419 residue amino acid sequence set forth in SEQ ID NO: 45.

At page 29, line 3, delete "this reading frame" and substitute therefor the reading frame specified in SEQ ID NOs: 32-33.

At page 31, line 20, after "ORF" please insert specified in SEQ ID NOs: 32 and 33.

In the claims:

Please amend claims 1, 3-5, 7, 18-19, 26-33 and 36-37; and add new claims 39-44 as shown below:

D5

1. (Three times amended) A host cell transformed or transfected with a polynucleotide (encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase), wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said polypeptide includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B)

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase.

D6

3. (Three times amended) A host cell transformed or transfected with a [nucleic acid encoding] polynucleotide comprising a nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, [said polypeptide including a domain defined by eight conserved

cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)) said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC).

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and wherein said polypeptide is capable of binding to Flt4EC.

D6 4. (Twice amended) A host cell according to claim 3 wherein said [nucleic acid] nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

5. (Three times amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising [a nucleic acid] an expression control sequence operatively linked to the nucleotide sequence that encodes [a polypeptide having] the amino acid sequence shown in SEQ ID NO: 33.

D7 7. (Twice amended) A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert. [plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)]

wherein said secreted polypeptide binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human

platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and

wherein said secreted polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P).

18. (Twice amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to [an] human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid [polynucleotide] lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. (Amended) A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of human Flt4 receptor tyrosine kinase.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human [VEGF-C] Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim [27] 1, wherein said host cell expresses said polynucleotide and produces a [mature] human [VEGF-C] protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to [a nucleotide] sequence that encodes said polypeptide.

D10 30. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes [a VEGF-homologous portion] eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A [polynucleotide] nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence [corresponding] identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion

D10 commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

D11 growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B). [conserved cysteines and having homology to vascular endothelial growth factor (VEGF)] and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

- 39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

D12 41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a

continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

D12 44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

REMARKS

I. History of claims and explanation of amendments.

A. Prosecution History

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In a responsive amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 were pending at the time the outstanding Office action was issued. In the outstanding Office action, claims 33-36 have been allowed, but claims 1, 3-5, 7, 11, 18-32, and 37-38 were rejected.

In the present amendment, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and 36-37; and add new claims 39-44. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

nucleotides 37 to 1086 represent the portion of SEQ ID NO: 32 that encodes the amino acid sequence specified in SED ID NO: 33.

The amendment to claim 5 to recite a vector comprising "an expression control sequence operatively linked" to a coding sequence finds support throughout the application, including at page 6, lines 28-30.

The amendment to claim 7 to specify that the host cell "secretes" the encoded polypeptide, and to specify that the secreted polypeptide binds to Flt4, is found throughout the application. For example, Example 11 (p. 28) of the application describes the expression and secretion into the cell culture medium of a polypeptide encoded by the insert of the deposited plasmid. The polypeptide bound Flt4 and stimulated Flt4 phosphorylation. New claims 39-40 are likewise supported by way of example (see Examples 6, 11, and 13, for example, teaching the use of eukaryotic/mammalian expression vectors and cell lines to express VEGF-C).

Claims 18 and 19 have been amended to recite "human" (i.e., "human Flt4"). This amendment is not intended to imply that polypeptides of the invention which bind to human Flt4 would not also bind to Flt4 proteins of other animals. Claim 18 also has been amended to recite "nucleic acid" instead of "polynucleotide." This amendment is not intended to alter the scope of the claim, but merely to use a term that has *ipsis verbis* antecedent basis in the preamble.

Claims 26 and 27 have been amended to recite "Flt4 ligand" instead of "VEGF-C." This amendment is not intended to diminish the scope of the claims, since VEGF-C is the name ascribed to an Flt4 ligand of the invention. See, e.g., specification at page 5, lines 17-19. Similarly, claim 28 has been amended to recite a human protein "that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase," instead of reciting human "VEGF-C."

Claim 28 also has been amended to delete the term "mature," which is believed to be unnecessary to define the invention, especially in view of the binding and molecular weight limitations of the claim.

The amendment to allowed claim 33 to recite "said polypeptide *having an amino acid sequence* consisting of a continuous portion of the sequence shown in SEQ ID NO: 33" is formal in nature and not intended to diminish the scope of the claim.

Likewise, the amendment to allowed claim 36 merely makes the language of the final step of the claim more closely parallel the language of the preamble. This amendment is formal in nature and not intended to diminish the scope of the claim.

New claim 41 finds support throughout the application as originally filed, including at page 23, lines 1-10, and claim 10 as originally filed.

New claim 42 is directed to a host cell transformed or transfected with a polynucleotide. The hybridization conditions recited in claim 42 are identical to those recited in claim 1 and find support, e.g., in Example 10 at page 27, lines 10-14. The recitation in claim 42 that the host cell "secretes" the expressed polypeptide finds support, e.g., in Example 11 (p. 28). The size and binding characteristics that are recited in new claim 42 find support throughout the application as originally filed, including in Example 5 and in original claims 8 and 9.

New claims 43-44 find support throughout the application as originally filed, including at page 6, lines 16-20. The specified terminal amino acids in claims 43-44 find support, e.g., at page 5, lines 27-34, and are the same terminal residues specified in allowed claim 33.

II. The rejection of claims 7 and 37 under 35 U.S.C. §112, first paragraph, was improper, and should be withdrawn.

Paragraphs 4 and 10-12 pertain to a rejection of claims 7 and 37 under 35 U.S.C. §112, first paragraph. The Patent Office indicates that it will withdraw the rejection if an appropriate statement is filed lifting all restrictions on the availability of a deposited plasmid, consistent with Budapest treaty. *The Applicants filed such a statement with their amendment dated November 26,*

1997. A copy of the statement is filed herewith. Thus, the objection to the specification and rejection should now be withdrawn.

III. The objection that a previous amendment introduced new matter should be withdrawn.

In paragraphs 4 and 10 of the Office action, the Patent Office objects to an amendment to introduce SEQ ID NO: 44 and 45 into the application, alleging that the amendment introduces new matter:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97321 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO: 45, as stated in the added sentence. SEQ ID NO: 45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO: 44

(Office action at p. 4; see also p. 2.)

The Applicants respectfully traverse.

The allegation that the previously-filed Rule 132 Alitalo declaration fails to state that the cDNA insert was derived from ATCC Deposit No. 97231 is incorrect. Paragraph 4 of the declaration identifies the plasmid by its ATCC accession number and paragraph 5 states, "Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45." Thus, the amendment to add SEQ ID NOs: 44-45 to the application had sufficient corroboration.

Notwithstanding the foregoing, the Applicants have adopted all of the Patent Office's suggestions to overcome the new matter objection. The Applicants have amended the application at page 29 to explain the relationship between the approximately 2.1 kb insert and the 1997 base pair sequence; the Applicants have clarified the DNA/encoded protein relationship between SEQ ID NO: 44 and 45; and the Applicants have filed herewith another declaration from Dr. Alitalo confirming that SEQ ID NOs: 44 and 45 represent nucleotide and deduced amino acid sequences of the deposited plasmid. Accordingly, the new matter objection should now be withdrawn.

IV. The rejection of claims 1, 18, 23-31, and 37-38 under §112, first paragraph, should be withdrawn.

In paragraphs 5 and 13 of the outstanding Office action, the Patent Office rejected claims 1, 18, 23-31, and 37-38 under §112, first paragraph, alleging that the specification does not reasonably enable the full scope of these claims. As its basis for rejection, the Patent Office alleges that neither the application nor the prior art enables one skilled in the art to use a polypeptide which binds to the Flt4 receptor and which does NOT stimulate tyrosine phosphorylation activity of the receptor. (Office action at pp. 5-7.) The Applicants respectfully traverse.

The present patent application teaches uses for polypeptides of the invention that bind, but fail to activate, the Flt4 receptor. For example, at page 7, lines 8-15, the application teaches that Flt4 ligand polypeptides of the invention can be labeled and used to identify their corresponding receptor *in situ*. Such labeled ligands can be used as detection or imaging agents, analogous to anti-Flt4 antibodies, to detect and/or image lymphatic vessels and high endothelial venules that express the Flt4 receptor on their surface. Such imaging/detection uses include uses for analyzing histochemical tissue sections. Those skilled in the art understand that the activity of binding to the extracellular domain of Flt4 is all that is required to make polypeptides effective for such uses. Stated differently, imaging a receptor with a labeled binding

agent does not require the labeled binding agent to activate the receptor. Such uses were discussed in an interview of March 24, 1998, in a related application (USSN 08/671,573), at which time Examiner Brown acknowledged that she had not considered such uses when entering the rejection. A similar rejection in the related application has now been withdrawn by the Patent Office.

The application also teaches that peptides which block the Flt4 receptor are useful as inhibitors to control endothelial cell proliferation and lymphangiomias. (See page 7, line 32, to page 8, line 2.) Persons skilled in the art understand that polypeptides that bind to the receptor but fail to activate the receptor can serve as competitive inhibitors. Thus, the application provides this additional use for polypeptides that bind Flt4 but fail to stimulate tyrosine phosphorylation of the receptor.

Because the present application teaches those skilled in the art "how to use a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation," the Patent Office's basis for rejection is unfounded. Accordingly, the rejection of claims 1, 18, 23-31, and 37-38 under §112, first paragraph, should be withdrawn.

V. The Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, should be withdrawn.

In paragraphs 15-22 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, alleging several bases why these claims were indefinite. The Applicants traverse-in-part and amend-in-part.

A. The rejection of claims 1, 3-5, 7, 26-29, and 37 relating to the term "a domain defined by eight conserved cysteine residues" should be withdrawn.

In paragraph 16 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 26-29, and 37, alleging that the term "a domain defined by eight conserved cysteine residues" was indefinite. The Applicants traverse-in-part and amend-in-part.

1. It is clear to what the eight residues are conserved.

As its first rationale for rejection, the Patent Office asserted, "It is unclear to what the eight residues are conserved." (Office action at p. 8.) The Applicants' amendments render this rationale moot. For example, claim 1 has been amended to recite, "wherein said polypeptide includes a domain defined by eight cysteine residues *that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).*" Claims 3, 7, and 37 have been amended similarly. The eight conserved cysteines are readily apparent to scientists skilled in the art. (See the alignment of VEGF, PDGF-A, and PDGF-B in Fig. 10A of the patent application, with conserved cysteines at positions 103, 130, 136, 139, 140, 147, 184, and 186.) Since the claim now recites "to what the eight residues are conserved," the basis for rejection is rendered moot.

2. The minimum limits of the domain are clear.

As its second rationale for rejection, the Patent Office asserted, "It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.)

The basis for the rejection is contrary to the plain language of the claims. If a domain is "defined by eight conserved cysteines," then it clearly is not "defined by a different parameter." Thus, the plain language of the claims demonstrates that this basis for rejection is improper, and that the minimum included portion of the encoded polypeptide is defined with particularity. Accordingly, the rejection should be withdrawn.²

² Moreover, the Applicants' amendments to claim 3 render this basis for rejection moot with respect to claims 3-5, because claim 3 no longer recites "domain defined by." Instead, claim 3 recites a "contiguous portion of SEQ ID NO: 33" that "includes" the eight conserved cysteines.

3. The objection to the term "homology" is now moot.

As a third basis for rejection the Patent Office alleged, "these claims are indefinite with respect to the term 'having homology to vascular endothelial growth factor.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF." (Office action at p. 8.)

The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term from claims 1, 3, 7, and 37, rendering this basis for rejection moot.

4. Conclusion

For the reasons set forth above, the rejection of claims 1, 3-5, 7, 26-29, and 37 should be withdrawn.

- B. The rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 with respect to the term "cysteine motifs of a Balbiani ring 3 protein" should be withdrawn.**

In paragraph 17 of the Office action, the Patent Office rejected claim 1, 3-5, 7, 11, 18-30, 32, and 37-38, alleging that the phrase "cysteine motifs of a Balbiani ring 3 protein" in claims 1, 3, 7, 18, 30, and 37 is indefinite. The Applicants respectfully traverse.

The Patent Office's first basis for rejection rests upon the two-part premise that "Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is." (Office action at p. 9.) Neither part of this premise is correct. The specification adequately defines the cysteine motifs of a Balbiani ring 3 protein (BR3P) at page 11, lines 16-25, citing two articles in the literature (both of record).³ The citation to literature in the art is

³ As discussed in paragraph 6 of the Rule 132 declaration of Dr. Alitalo dated November 26, 1997, BR3P cysteine motifs are quite distinctive in character (Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys) and occur at least four times in the

more than adequate to describe that which is already known in the art. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986) (It is axiomatic that patent applications need not contain, and preferably omit, that which is well known in the art.).⁴

Moreover, even if the specification lacked the description at page 11, the fact remains that the characteristic BR3P cysteine motif was within the knowledge of those skilled in the art at the time of filing, such that one skilled in the art could determine whether or not a polypeptide contained a domain characterized by one or more BR3P cysteine motifs. See M.P.E.P. § 2164.08 ("Not everything necessary to practice the invention need be disclosed. In fact, what is well-known is best omitted.") Thus, the patent application contains sufficient definition of cysteine motifs of a BR3P protein for the reader skilled in the art. See, e.g., *In re Moore*, 169 U.S.P.Q. 236, 238 (CCPA 1971) (Claim language must not be analyzed in a vacuum, "but always in light of the teachings of the prior art and of the particular application disclosure as it would be interpreted by one possessing the ordinary level of skill in the pertinent art.").

The Patent Office's second basis for rejection alleges that "it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter." (Office action at p. 8.) The Applicants respectfully submit that the plain language of the claims state that the claimed polypeptides lack portions *defined by the cysteines*, and that no reasonable alternative interpretation exists. For example, amended claim 1 recites, in

carboxy terminal portion of the VEGF-C precursor polypeptide (see, e.g., SEQ ID NO: 44, Cys residues at positions 280, 291, 293, 295; residues 304, 315, 317, 319; residues 328, 339, 341, and 343; and residues 347, 358, 360, and 362). The application depicts the VEGF-C precursor amino acid sequence, and the distinctive BR3P motifs in the carboxy-terminus would be readily apparent to the reader skilled in the art.

⁴ Notwithstanding the accepted practice of omitting that which is well known in the art, the Applicants will amend the specification to include an excerpt from the cited Dignam and Case article, if the Patent Office requests.

pertinent part, that the polypeptide "lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P)." There is no ambiguity to determining whether or not a protein's amino acid sequence includes or lacks one or more "Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys" sequences.

For all of these reasons, the rejection of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 should be withdrawn.

- C. The rejection of claims 1, 26-29, and 37 with respect to the term "high affinity" has been rendered moot.

In paragraph 18 of the Office action, the Patent Office rejected claims 1 and claims 26-29, and 37 which depend therefrom, alleging that the claims were indefinite with respect to the term "high affinity" recited in claim 1: "The term 'high affinity' is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered 'high affinity.' It is suggested that the claims be amended to recite a particular range of K_d ." (Office action at p. 8.)

The Applicants respectfully submit that the term "high affinity" is not indefinite to a person of ordinary skill in the art in view of the teachings of the application and the art to which the invention pertains. Notwithstanding this fact and solely to expedite allowance, the Applicants have amended claim 1 to delete the allegedly indefinite term, rendering this basis for rejection moot. The subject matter of the claim is adequately defined by the limitations that remain after this amendment.

- D. The rejection of claims 1, 3-5, 7, 26-30, and 37 with respect to the term "including" should be withdrawn.

In paragraph 19 of the outstanding Office action, the Patent Office rejected claims 1, 3-5, 7, 26-30, and 37, alleging that the term "including" was indefinite because "it is unclear whether 'including' is equivalent to the open language 'comprising' or the closed language 'consisting of.'" (Office action at p. 9.) The Applicants respectfully traverse. The term "including" is unequivocally interpreted as open claim language, synonymous with the term

"comprising." See M.P.E.P. §2111.03. Accordingly, this rejection should be withdrawn.

- E. The rejection of claims 3, 5, 18, 24-25, and 30-31 for lack of antecedent basis has been rendered moot.

In paragraph 20 of the outstanding Office action, the Patent Office rejected claims 3, 5, 18, 24-25, and 30-31, alleging that the term "said polynucleotide" as recited in the claims lacks antecedent basis. The Applicants have amended claim 3 to provide *ipsis verbis* antecedent basis for the term "said polynucleotide," thereby rendering the rejection moot with respect to claim 3 and also claim 5 which depends from claim 3.

The Applicants have amended claims 18 and 30-31 to recite "nucleic acid" instead of "polynucleotide." This substitution of terminology renders moot the rejection of claims 18, 24-25, and 30-31. The term "nucleic acid" in the amended claims has *ipsis verbis* antecedent basis support.

For these reasons, the rejection of claims 3, 5, 18, 24-25, and 30-31 has been rendered moot, and should be withdrawn.

- F. The rejection of claim 30 has been rendered moot.

In paragraph 21 of the Office action the Patent Office alleged, "Claim 30 is indefinite with respect to the term 'VEGF-homologous portion.' It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF" (Office action at p. 9.) The Applicants respectfully submit that this phrase is clear and that "similarity" and "common evolutionary origin" are not incompatible concepts. However, solely to expedite allowance, the Applicants have deleted the allegedly indefinite term, rendering this basis for rejection moot. Accordingly, the rejection of claim 30 should be withdrawn.

G. The rejection of claim 32 has been rendered moot.

In paragraph 22 of the Office action, the Patent Office alleged, "Claim 32 is indefinite with respect to an amino acid sequence 'corresponding to' another amino acid sequence. It is unclear whether 'corresponding to' means that the amino acid sequence is identical or not." (Office action at p. 9.) Solely to expedite allowance, the Applicants have substituted the term "identical to" for the term "corresponding to" in claim 32, rendering this rejection moot.

H. Conclusion.

For all of the foregoing reasons, the Patent Office's rejections of claims 1, 3-5, 7, 11, 18-30, 32, and 37-38 under 35 U.S.C. §112, second paragraph, should now be withdrawn.

VI. Status update relating to priority applications.

The 1994 priority application has now issued as U.S. Patent No. 5,776,712. The Applicants wish to apprise the Examiner that prosecution has been suspended in U.S.S.N. 08/510,133 because "A reference relevant to the examination of this application may soon become available."

VII. Summary

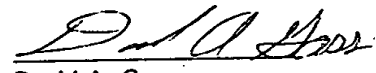
The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: July 23, 1998


David A. Gass
Registration No. 38,153

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. (Amended) A host cell according to claim 1 that expresses a naturally occurring Flt4 ligand protein encoded by said polynucleotide.

27. (Amended) A host cell according to claim 1 that expresses a human Flt4 ligand protein encoded by said polynucleotide.

28. (Amended) A host cell according to claim 1, wherein said host cell expresses said polynucleotide and produces a human protein that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase, said protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. (Amended) A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

30. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. (Amended) A nucleic acid according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. (Amended) A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. (Amended) A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating phosphorylation of Flt4 receptor tyrosine kinase.

37. (Amended) A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

39. A method according to claim 38 wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

40. A eukaryotic host cell according to claim 1 or 3 that secretes said polypeptide.

41. A nucleic acid according to claim 30 wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

42. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

43. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

44. A purified and isolated nucleic acid according to claim 43 wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.



GAU 16
#2
PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|------------------------|
| Applicant(s): Alitalo et al. |) | Title: RECEPTOR LIGAND |
| Serial No: 08/585,895 |) | Group Art Unit: 1646 |
| Filed: January 12, 1996 |) | Examiner: Saoud |
| |) | |
| |) | |
| |) | |

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

RECEIVED

AUG 4 1998

*Assistant Commissioner for Patents
Washington, D.C. 20231*

RECEIVED
SERVICE CENTER

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
3. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
4. Check in the amount of \$55.00 in payment of fee for extension of time; and
5. Check in the amount of \$159.00 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 23, 1998, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

☒ Small entity status has been established and is still effective.

2. **Extension of Time**

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | FEE FOR LARGE ENTITY | | FEE FOR SMALL ENTITY | |
|-----------------------|----------------------|------------|----------------------|----------|
| One Month | | \$110.00 | X | \$55.00 |
| Two Months | | \$400.00 | | \$200.00 |
| Three Months | | \$950.00 | | \$475.00 |
| Four Months | | \$1,510.00 | | \$755.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00

RECEIVED

AUG 4 1998

Matrix Corporation
SERVICE CENTER

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

| | | | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
|---|----------------------------------|---------------------------------|----|---------------|--------------|----------------|---------------------------|----------------|
| | Claims Remaining After Amendment | Highest No. Previously Paid For | | Present Extra | Rate | Additional Fee | Rate | Additional Fee |
| TOTAL | 40 | MINUS | 33 | 7 | X11 = | \$77 | X22 = | \$ |
| INDEP. | 7 | MINUS | 5 | 2 | X41 = | \$82 | X82 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | | | + 135 = | | + 270 = | \$ |
| TOTAL ADDITIONAL FEE | | | | | | \$159 | OR | \$ |

4. Method of Payment of Fees

☒ Attached are checks in the amount of \$55.00 and \$159.00.

☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. Deposit Account and Refund Authorization

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

July 23, 1998



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|------------------------|
| Applicant(s): Alitalo et al. |) | Title: RECEPTOR LIGAND |
| Serial No: 08/585,895 |) | Group Art Unit: 1646 |
| Filed: January 12, 1996 |) | Examiner: Saoud |
| |) | |
| |) | |

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED

AUG 4 1998

CUSTOMER
SERVICE CENTER

Sir:

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4. Check in the amount of \$55.00 in payment of fee for extension of time; and
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David A. Gass

1. **Small Entity Status**

☒ Small entity status has been established and is still effective.

2. **Extension of Time**

☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | FEE FOR LARGE ENTITY | | FEE FOR SMALL ENTITY | |
|-----------------------|----------------------|------------|----------------------|----------|
| | | | | |
| One Month | | \$110.00 | X | \$55.00 |
| Two Months | | \$400.00 | | \$200.00 |
| Three Months | | \$950.00 | | \$475.00 |
| Four Months | | \$1,510.00 | | \$755.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$55.00

3. Fee for Claims

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

| | | | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
|---|----------------------------------|---------------------------------|----|---------------|--------------|----------------|---------------------------|----------------|
| | Claims Remaining After Amendment | Highest No. Previously Paid For | | Present Extra | Rate | Additional Fee | Rate | Additional Fee |
| TOTAL | 40 | MINUS | 33 | 7 | X11 = | \$77 | X22 = | \$ |
| INDEP. | 7 | MINUS | 5 | 2 | X41 = | \$82 | X82 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | | | +135 = | | +270 = | \$ |
| TOTAL ADDITIONAL FEE | | | | | | \$159 | OR | \$ |

4. Method of Payment of Fees

- ☒ Attached are checks in the amount of \$55.00 and \$159.00.
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
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Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
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6300 Sears Tower
233 South Wacker Drive
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(312) 474-6300

By:


David A. Gass
Reg. No: 38,153

July 23, 1998

Jul. 23. 1998 5:15PM MARSHALL, OTOOLE

No. 8729 P. 1/4
From: 0819

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

ATTORNEYS AT LAW
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402
(312) 474-6300
FAX: (312) 474-0448

July 23, 1998

FACSIMILE TRANSMITTAL SHEET

TO: Examiner Saoud - Group Art Unit: 1646
c/o U.S. Patent and Trademark Office
(703) 308-0294
U.S. Serial No. 08/585,895

CLIENT NO: 28967
MATTER NO: 33072
COUNTRY CODE: US

FROM: David A. Gass
Marshall, O'Toole

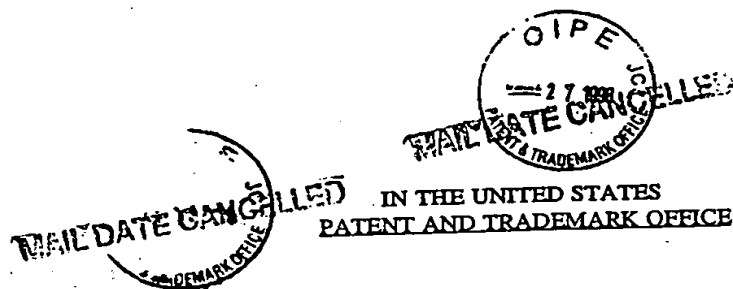
PAGES (INCLUDING THIS PAGE): 4

PLEASE CONFIRM RECEIPT: No.

MESSAGE: The attached declaration, which is a duplicate of a declaration filed November 26, 1997, was referenced in amendment papers filed today by first class mail in the above matter, but may have been inadvertently omitted.

Please contact Lisa Richard at (312) 474-6819 if you do not receive all of the pages in good condition.

The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.



PATENT
28967/33072



In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


Title: RECEPTOR LIGAND

Art Unit: 1646

Examiner: Saoud

I hereby certify that this paper is
deposited with the United States Postal
Service as first class mail, postage
prepaid, in an envelope addressed to:
Assistant Commissioner for Patents
Washington, D.C. 20231, on this date:

Dated: July 23, 1998


David A. Gass

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

I, Kari Alitalo, do hereby declare and state as follows:

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated March 24, 1998, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

2. I understand that sequences identified as SEQ ID NOS: 44 and 45 were added to the patent application by an amendment dated November 26, 1997, and entered by the Patent Office on December 1, 1997. Copies of those two sequences are appended hereto. I understand that, at the time of the amendment, SEQ ID NOS: 44 and 45 were identified as a nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the American Type Culture Collection (ATCC) as plasmid pFLT4-L and that is cross-referenced in the patent application at pages 28-29. I understand that the Patent Office has objected to the amendment to introduce these two sequences into the patent application on the

basis that such an amendment "introduces new matter into the disclosure." The Patent Office's basis for this allegation was as follows:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "the approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44."

(Office action dated March 24, 1998, at paragraph 10.)

3. I confirm that our laboratory sequenced the insert of the same plasmid that was designated pFLT4-L and that was deposited with the ATCC as ATCC Deposit No. 97231 and that is referred to at pages 28-29 of the patent application. The nucleotide sequence of the insert of this plasmid (ATCC Deposit No. 97231) includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44 as appended hereto and added to the patent application in the amendment dated November 26, 1997. The 419 residue amino acid sequence set forth in SEQ ID NO: 45 (as appended hereto and added to the patent application) is deduced from the sequence set forth in SEQ ID NO: 44.

4. The insert of plasmid pFLT4-L (ATCC Deposit No. 97231) contains additional (non-coding) sequence adjacent to the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The apparent size discrepancy between the approximately 2.1 kb size of the insert (as estimated by agarose gel electrophoresis analysis) and the 1997 nucleotides of sequence as set forth in SEQ ID NO: 44 is explained by the existence of this additional non-coding sequence in the plasmid insert.

Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and

the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

July 17, 1998
Date

Kari Alitalo
Kari Alitalo

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

| | |
|---|---------|
| CCCCCCCCGC CTCTCCAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGCC | 60 |
| CTCGCTTCAC CTCGGGGGCT CCGAATGCGG GGAGCTCGGA TGTCCGGTTT CCTGTGAGGC | 120 |
| TTTACCTGA CACCCGCCGC CTTCGCCCG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG | 180 |
| GGAACGCGGA GCCCGGACC CGTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG | 240 |
| GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGG GCCCGCGCCC | 300 |
| CCACCCCTGC CCCC GCCAGC GGACCGGTCC CCCACCCCG GTCTTCCAC C ATG CAC | 357 |
| | Met His |
| | 1 |
| TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG | 405 |
| Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu | |
| 5 10 15 | |
| CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC | 453 |
| Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser | |
| 20 25 30 | |
| GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT | 501 |
| Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala | |
| 35 40 45 50 | |
| TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA | 549 |
| Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val | |
| 55 60 65 | |
| GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG | 597 |
| Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys | |
| 70 75 80 | |
| TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC | 645 |
| Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn | |
| 85 90 95 | |

| | |
|---|------|
| CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT | 693 |
| Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr | |
| 100 105 110 | |
| AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA | 741 |
| Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln | |
| 115 120 125 130 | |
| TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC | 789 |
| Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val | |
| 135 140 145 | |
| GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT | 837 |
| Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys | |
| 150 155 160 | |
| GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG | 885 |
| Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr | |
| 165 170 175 | |
| AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA | 933 |
| Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln | |
| 180 185 190 | |
| GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA | 981 |
| Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg | |
| 195 200 205 210 | |
| TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA | 1029 |
| Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg | |
| 215 220 225 | |
| CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC | 1077 |
| Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr | |
| 230 235 240 | |
| TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT | 1125 |
| Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala | |
| 245 250 255 | |
| CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT | 1173 |
| Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp | |
| 260 265 270 | |
| GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC | 1221 |
| Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr | |
| 275 280 285 290 | |
| TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC | 1269 |
| Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro | |
| 295 300 305 | |
| CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA | 1317 |
| His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys | |
| 310 315 320 | |

| | |
|---|------|
| CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335 | 1365 |
| TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350 | 1413 |
| CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370 | 1461 |
| TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385 | 1509 |
| CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400 | 1557 |
| GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415 | 1605 |
| AGC TAAGATTGTA CTGTTTTCCTA GTTCATCGAT TTTCTATTAT GGAAAACCTGT Ser | 1658 |
| GTGCCCACAG TAGAACTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA | 1718 |
| CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC | 1778 |
| TGCAAAAGGC CTCTGTAAA GACTGGTTTT CTGCCAATGA CCAACAGCC AAGATTTTCC | 1838 |
| TCTGTGATT TCITTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT | 1898 |
| GCATTCAITTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC | 1958 |
| ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT | 1997 |

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 419 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | His | Leu | Leu | Gly | Phe | Phe | Ser | Val | Ala | Cys | Ser | Leu | Leu | Ala | Ala |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
| Ala | Leu | Leu | Pro | Gly | Pro | Arg | Glu | Ala | Pro | Ala | Ala | Ala | Ala | Ala | Phe |
| | | | 20 | | | | | 25 | | | | | | 30 | |

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
 35 40 45
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
 50 55 60
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
 65 70 75 80
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
 85 90 95
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335

Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
405 410 415

Gln Met Ser



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| SERIAL NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|---------------|-------------|-----------------------|---------------------|
| 08/585,895 | 01/12/98 | ALFALU | K 28113/33072 |

HM11/1009
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6000 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, IL 60606-6402

EXAMINER

ART.UNIT. PAPER NUMBER

27
10/08/98

DATE MAILED:

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents

Applicant's response filed 27 July 1998 has been received. However, a reference relevant to the examination of this application may soon become available. *Ex parte* prosecution is SUSPENDED INDEFINITELY from the date of this letter. Applicant should feel free to make an inquiry as to the status of the application if needed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Lila Feisee, can be reached on (703) 308-2731. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Christine Saoud, Ph.D.
October 6, 1998

JOHN ULM
PRIMARY EXAMINER
GROUP 1800



Class 1646

PATENT
Attorney Docket No. 28967133072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

JUL 29 1999

TECH CENTER 1000-00

In the Application of: Kari Alitalo

and Vladimir Joukov

Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: Saoud, C.

) I hereby certify that this paper and
) the documents referred to as
) enclosed herewith are being
) deposited with the United States
) Postal Service as First Class Mail,
) postage prepaid, in an envelope
) addressed to: Assistant
) Commissioner for Patents,
) Washington, DC 20231, on this
) date:

) July 26, 1999

) Jill E. Uhl
) Jill E. Uhl

) Reg. No.: 43,213

) Attorney for Applicants

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents,
together with a copy of each listed document. The Applicants respectfully request that
these documents be made of record and considered in the above-identified application.

Documents A3-A6 are U.S. priority documents of published PCT
applications that are now publically available from WIPO.

Documents B9-B11, C117, C119, and C154-C157 were identified by the
European Patent Office in an International Search Report for a related PCT application. A

copy of the search report is also attached hereto.

Documents C120-C153 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

July 26, 1999

By:

Jill E. Uhl
Jill E. Uhl
Registration No.: 43,213
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT

Attorney Docket No. 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo

and Vladimir Joukov

Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: Saoud, C.

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) date:

) July 26, 1999

) Jill E. Uhl
) Jill E. Uhl

) Reg. No.: 43,213

) Attorney for Applicants

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MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

July 26, 1999

By:

Jill E. Uhl
Jill E. Uhl
Registration No.: 43,213
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

FILE COPY

#28

SHEET 1 of 5

| | | | |
|--|--|---------------------------------|--------------------------|
| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo, K. et al. | |
| | | Filing Date January 12, 1996 | Group 1646 |

| U.S. PATENT DOCUMENTS | | | | | | | |
|-----------------------|----|-----------------|------------|---|-------|----------|----------------------------|
| *Examiner Initials | | Document Number | Issue Date | Name | Class | Subclass | Filing Date If Appropriate |
| ck | A3 | 08/207,550 | none | Jing-Shan Hu and Liang Cao | | | 03/08/94 |
| ck | A4 | 08/465,968 | none | Crain Rosen, Jing-Shan Hu and Liang Cao | | | 06/06/95 |
| ck | A5 | 60/003,491 | none | James Lee and William Wood | | | 09/08/95 |
| ck | A6 | 08/554,374 | none | Lyman, S. | | | 11/08/95 |

| FOREIGN PATENT DOCUMENTS | | | | | | | | |
|--------------------------|-----|-----------------|------------------|---------|-------|----------|-------------|----|
| *Examiner Initials | | Document Number | Publication Date | Country | Class | Subclass | Translation | |
| | | | | | | | Yes | No |
| ck | B8 | 0 506 477 A1 | 03/27/92 | EP | | | | |
| ck | B9 | 97/05250 A | 02/13/97 | WO | | | | |
| ck | B10 | 97/09427 A | 03/13/97 | WO | | | | |
| ck | B11 | 97/17442 A | 05/15/97 | WO | | | | |

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|---|--------------------------------|
| EXAMINER <i>C. Saoud</i> | DATE CONSIDERED <i>3/28/00</i> |
| *EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. | |

SHEET 2 of

| | | | |
|--|--|--|--------------------------|
| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
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| | | Filing Date January 12, 1996 | Group 1646 |

| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
|--|------|---|
| u | C117 | Achen, M.G. <i>et al.</i> , "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," <i>Proceedings of the National Academy of Science, USA</i> , 95:548-553 (January, 1998). |
| | C118 | Adams, M.D. <i>et al.</i> , "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," <i>Nature</i> , 377(6547 Supplement):3-174 (September, 1995). |
| | C119 | Cohen, T. <i>et al.</i> , "VEGF ₁₂₁ , A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," <i>Journal of Biological Chemistry</i> , 270(19):11322-11326 (May 12, 1995). |
| | C120 | Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'," Hillier, L. <i>et al.</i> , Dated 14-May-1997 |
| | C121 | Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF_MOUSE Q00731 VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997 |
| | C122 | Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996 |
| | C123 | Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996 |
| | C124 | Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'," Deposited by Hillier, L. <i>et al.</i> Dated 17-May-1997 |
| | C125 | Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'," Deposited by Hillier, L. <i>et al.</i> Dated 14-Feb-1996 |
| u | C126 | Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997 |

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| EXAMINER C. Saoud | DATE CONSIDERED 3/28/00 |
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| | | | |
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| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo, K. <i>et al.</i> | |
| | | Filing Date January 12, 1996 | Group 1646 |

| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
|--|------|--|
| ✓ | C127 | Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Aug-1996 |
| | C128 | Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995 |
| | C129 | Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997 |
| | C130 | Genbank H05134, "y185b08.s1 Homo sapiens cDNA clone 44993 3'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995 |
| | C131 | Genbank, AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997 |
| | C132 | Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to similar to vascular endothelial growth factor," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997 |
| | C133 | Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995 |
| | C134 | Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997 |
| | C135 | Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995 |
| | C136 | Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995 |
| | C137 | Genbank AA478766, "zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997 |
| ✓ | C138 | Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996 |

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|---|----------------------------|
| EXAMINER C. Saoud | DATE CONSIDERED 3/28/00 |
| *EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. | |

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| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo, K. <i>et al.</i> | |
| | | Filing Date January 12, 1996 | Group 1646 |

| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
|--|------|---|
| CA | C139 | Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996 |
| | C140 | Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995 |
| | C141 | Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 16-Mar-1995 |
| | C142 | Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996 |
| | C143 | Genbank N42374, "yy15d11.r1 Homo sapiens cDNA clone 271317 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996 |
| | C144 | Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995 |
| | C145 | Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995 |
| | C146 | Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3 PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 14-May-1997 |
| | C147 | Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'," Deposited by Hillier, L. <i>et al.</i> Dated 07-Jun-1995 |
| | C148 | Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'," Deposited by Hillier, L. <i>et al.</i> Dated 23-Jun-1995 |
| | C149 | Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Mar-1995 |
| CA | C150 | Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated 01-Oct-1996 |

| | |
|---|----------------------------|
| EXAMINER C. Saoud | DATE CONSIDERED 3/28/96 |
| *EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. | |

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| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo, K. <i>et al.</i> | |
| | | Filing Date January 12, 1996 | Group 1646 |

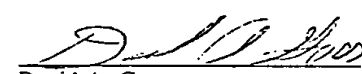
| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
|--|------|--|
| OK | C151 | Genbank N82975, "TgESTzy53h10.r1 TgRH Tachyzoite cDNA Toxoplasma gondii cDNA clone tgzy53h10.r1 5'," Deposited by Hehl, A. <i>et al.</i> Dated 10-Sep-1997 |
| | C152 | Genbank AA285997, "vb88h06.r1 Soares mouse 3NbMS Mus musculus cDNA clone 764123 5'," Deposited by Marra, M. <i>et al.</i> Dated 09-Apr-1997 |
| | C153 | Genbank AA549856, "0929m3 gmbPfHB3.1, G. Roman Reddy Plasmodium falciparum genomic clone 0929m," Deposited by Dame, J.B. <i>et al.</i> Dated 11-Aug-1997 |
| | C154 | Jeltsch, M. <i>et al.</i> , "Hyperplasia of Lymphatic Vessels in VEGF-C Transgenic Mice," <i>Science</i> , 276:1423-1425 (May, 1997). |
| | C155 | Joukov, V. <i>et al.</i> , "Proteolytic Processing Regulates Receptor Specificity and Activity of VEGF-C," <i>EMBO Journal</i> , 16(13):3898-3911 (June, 1997). |
| | C156 | Joukov, V. <i>et al.</i> , "A Recombinant Mutant Vascular Endothelial Growth Factor-C that has Lost Vascular Endothelial Growth Factor Receptor-2 Binding, Activation, and Vascular Permeability Activities," <i>Journal of Biological Chemistry</i> , 273(12):6599-6602 (March 20, 1998). |
| OK | C157 | Lee, J. <i>et al.</i> , "Vascular Endothelial Growth Factor Related Protein (vrp): A Ligand and Specific Activator of the Tyrosine Kinase Receptor Flt4," EMBL Sequence Data Library, XP002066361, accession no. U4142. Dated 10-Jan-1996 |

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37 1894
PATENT
Attorney Docket No: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------------------|---|--|
| In the Application of: Kari Alitalo |) | I hereby certify that this paper and the |
| |) | documents referred to as enclosed |
| and Vladimir Joukov |) | herewith are being deposited with the |
| |) | United States Postal Service as First |
| Serial No.: 08/585,895 |) | Class Mail, postage prepaid, in an |
| |) | envelope addressed to: Assistant |
| Filed: January 12, 1996 |) | Commissioner for Patents, |
| |) | Washington, DC 20231, on this date: |
| For: RECEPTOR LIGAND |) | |
| |) | October 26, 1999 |
| Group Art Unit: 1646 |) | |
| |) |  |
| Examiner: Saoud, C. |) | David A. Gass |
| |) | Reg. No.: 38,153 |
| |) | Attorney for Applicants |

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The applicants request that the documents listed on the attached Form PTO-1449 be made of official record and considered by the Examiner in the above-identified application.

Pursuant to 37 C.F.R. §1.98(d), copies of all listed documents are not enclosed because they were cited in a prior application (U.S. Serial No. 08/510,133, filed August 1, 1995) that is presently relied upon herein for an earlier filing date. However, copies of these documents will be resubmitted at the Examiner's request.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

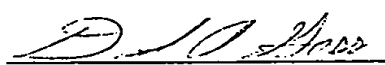
Pursuant to 37 C.F.R. §1.97(e)(2), the listed documents were not known to the applicants or to any individual designated in §1.56 (c) as issued U.S. patents more than three months prior to the filing of this Supplemental Information Disclosure Statement, because U.S. Patent No. 5,932,540 (document A7) issued on August 3, 1999 and U.S. Patent No. 5,935,820 (document A8) issued on August 10, 1999. Consequently, this Supplemental Information Disclosure Statement should be considered by the Patent Office without payment of a fee. However, please charge any necessary fees due in connection with this Supplemental Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

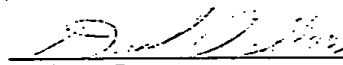
October 26, 1999

By:


David A. Gass
Reg. No.: 38,153

PATENT
Attorney Docket No. 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------------------|---|--|
| In the Application of: Kari Alitalo |) | I hereby certify that this paper and the |
| |) | documents referred to as enclosed |
| and Vladimir Joukov |) | herewith are being deposited with the |
| |) | United States Postal Service as First |
| Serial No.: 08/585,895 |) | Class Mail, postage prepaid, in an |
| |) | envelope addressed to: Assistant |
| Filed: January 12, 1996 |) | Commissioner for Patents, |
| |) | Washington, DC 20231, on this date: |
| For: RECEPTOR LIGAND |) | |
| |) | October 26, 1999 |
| Group Art Unit: 1646 |) | |
| |) | |
| Examiner: Saoud, C. |) |  |
| |) | David A. Gass |
| |) | Reg. No.: 38,153 |
| |) | Attorney for Applicants |

**SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The applicants request that the documents listed on the attached Form PTO-1449 be made of official record and considered by the Examiner in the above-identified application.

Pursuant to 37 C.F.R. §1.98(d), copies of all listed documents are not enclosed because they were cited in a prior application (U.S. Serial No. 08/510,133, filed August 1, 1995) that is presently relied upon herein for an earlier filing date. However, copies of these documents will be resubmitted at the Examiner's request.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

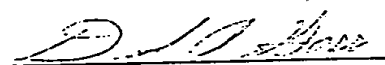
Pursuant to 37 C.F.R. §1.97(e)(2), the listed documents were not known to the applicants or to any individual designated in §1.56 (c) as issued U.S. patents more than three months prior to the filing of this Supplemental Information Disclosure Statement, because U.S. Patent No. 5,932,540 (document A7) issued on August 3, 1999 and U.S. Patent No. 5,935,820 (document A8) issued on August 10, 1999. Consequently, this Supplemental Information Disclosure Statement should be considered by the Patent Office without payment of a fee. However, please charge any necessary fees due in connection with this Supplemental Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

October 26, 1999

By:


David A. Gass
Reg. No.: 38,153

FILE COPY

#2
SHEET 1 of 1

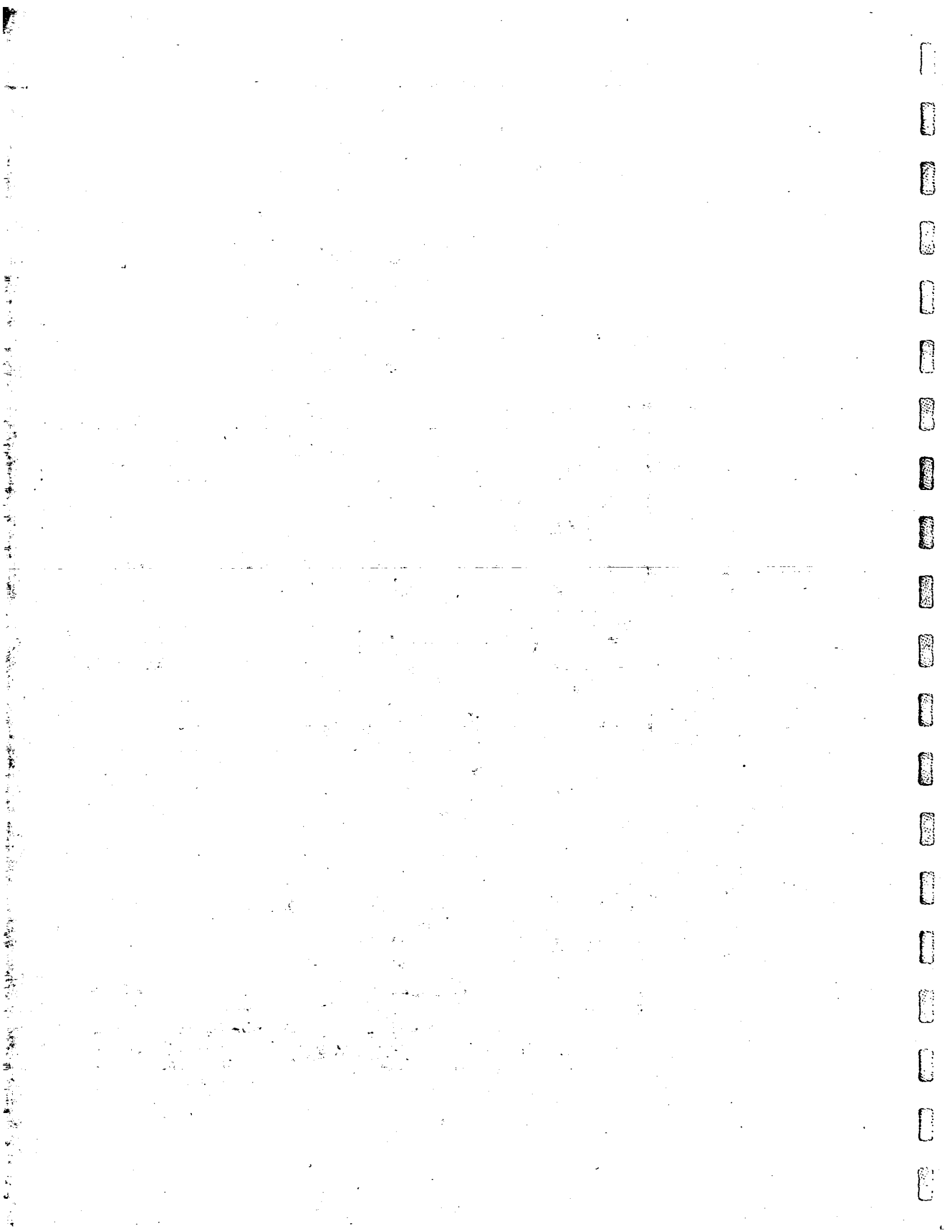
| | | | |
|---|--|---------------------------------|--------------------------|
| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT 3 (Use several sheets if necessary) | | Applicant Alitalo, K. et al. | |
| | | Filing Date January 12, 1996 | Group 1646 |

| U.S. PATENT DOCUMENTS | | | | | | | |
|-----------------------|----|--------------------|---------------|----------------------------|----------------|-----------------|----------------------------------|
| *Examiner Initials | | Document Number | Issue Date | Name | Class | Subclass | Filing Date If Appropriate |
| OK | A7 | 5,932,540 | 08/03/99 | Jing-Shan Hu <i>et al.</i> | 514 | 2 | |
| OK | A8 | 5,935,820 | 08/10/99 | Jing-Shan Hu <i>et al.</i> | 435 | 69.4 | |

| FOREIGN PATENT DOCUMENTS | | | | | | | | |
|--------------------------|--|--------------------|---------------------|---------|-------|----------|-------------|----|
| *Examiner Initials | | Document Number | Publication Date | Country | Class | Subclass | Translation | |
| | | | | | | | Yes | No |
| | | | | | | | | |

| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
|--|--|--|
| | | |

| | |
|---|--------------------------------|
| EXAMINER <i>C. Saoud</i> | DATE CONSIDERED <i>3/28/00</i> |
| *EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. | |





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|-----------------|-------------|----------------------|---------------------|
| 08/585,895 | 01/12/96 | ALITALO | K 28113/33072 |

HM22/0404
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

SAOUD, C

| ART UNIT | PAPER NUMBER |
|----------|--------------|
|----------|--------------|

1646

2c

DATE MAILED: 04/04/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.
08/585,895

Applicant(s)

Alitalo et al.

Examiner

Christine Saoud

Group Art Unit
1646

- ☐ Responsive to communication(s) filed on _____
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.
- A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☒ Claim(s) 1, 3-5, 7, 11, and 18-44 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claims _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- *Certified copies not received: _____
- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of References Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 28 and 29
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

— SEE OFFICE ACTION ON THE FOLLOWING PAGES —

Application/Control Number: 08/585,895

Page 2

Art Unit: 1646

DETAILED ACTION

Response to Amendment

1. Claims 1, 3-5, 7, 18, 19, 26-33, and 36-37 have been amended and claims 39-44 have been added as requested in the amendment of paper #26, filed 27 July 1998. Claims 1, 3-5, 7, 11, and 18-44 are pending in the instant application.
2. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.
3. Any objection or rejection of record which is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.
4. Applicant's arguments filed 27 July 1998 have been fully considered, however, in light of the new grounds of rejection below, the arguments are not found to be relevant and therefore, have not been addressed.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

Application/Control Number: 08/585,895

Page 3

Art Unit: 1646

make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1, 37, and 42 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1 and 42 and dependent claim 37 are directed to subject matter of a polynucleotide that hybridizes to a DNA under specific conditions which are recited in the claims, wherein the polynucleotide encodes a protein which has particular structural and functional features. In making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, it is necessary to understand what Applicant has possession of and what Applicant is claiming. From the specification, it is clear that Applicant has possession of a nucleic acid molecule which encodes a protein which has the amino acid sequence of SEQ ID NO:33. This nucleic acid molecule has a nucleic acid sequence of SEQ ID NO:32 and is contained within plasmid pFLT4-L (ATCC deposit #97231). The subject matter which is claimed is described above. First, a determination of the level of predictability in the art must be made in that whether the level of skill in the art leads to a predictability of structure; and/or whether teachings in the application or prior art lead to a predictability of structure. The claims are directed to host cells which are transfected with a polynucleotide which encodes a polypeptide, wherein the polynucleotide hybridizes to a DNA of SEQ ID NO:32 under a specific set of hybridization conditions. First, the claims are not limited to polynucleotide molecules

Art Unit: 1646

encoding a protein with a specific amino acid sequence. The claims only require the nucleic acid molecule to encode a polypeptide which belongs to the VEGF/PDGF family (implied by the recitation of the 8 cysteine domain) and which is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The specification only describes a single polypeptide from a human and fails to teach or describe any other polypeptide which has the structural and functional characteristics recited in the claims. The breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. There is a lack of guidance or teaching regarding structure and function because there is only a single example provided in the specification and because there is no guidance found in the prior art. The claims include polynucleotides which share some sequence similarity to the disclosed polynucleotide which encodes the polypeptide of SEQ ID NO:33, however, this sequence similarity is not sufficient to provide the function of encoding a polypeptide which binds to the Flt4 receptor tyrosine kinase.

Next in making a determination of whether the application complies with the written description requirement of 35 U.S.C. 112, first paragraph, each claimed species and genus must be evaluated to determine whether there is sufficient written description to inform a skilled artisan that applicant was in possession of the claimed invention at the time the application was filed. With this regard, the instant application fails to provide a written description of the species or the genus which are encompassed by the instant claims except for the nucleic acid of SEQ ID NO:32. The specification does not provide a complete structure of those polynucleotides which encode a

Application/Control Number: 08/585,895

Page 5

Art Unit: 1646

polypeptide as described in the claims and hybridize to the recited sequence under the recited stringency conditions of the claims. The claims also fail to recite other relevant identifying characteristics (physical and/or chemical and/or functional characteristics coupled with a known or disclosed correlation between function and structure) sufficient to describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. The specification fails to provide a representative number of species for the claimed genus (those polynucleotides which hybridize to SEQ ID NO:32 under the recited stringency conditions) because the claims are directed to those polynucleotides which encode a polypeptide having a conserved cysteine domain and which binds to the human Flt4 receptor tyrosine kinase, which encompasses different species and variants and the specification teaches one embodiment. Therefore, the claims are directed subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim Rejections - 35 USC § 102

7. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who

Art Unit: 1646

has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

8. Claims 1, 3-5, 7, 11, and 18-44 are rejected under 35 U.S.C. 102(e) as being anticipated by Hu et al. (U.S. Pat. No. 5,935,820).

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

Allowable Subject Matter

9. It is noted that some of the claims appear to be directed to polynucleotides encoding a polypeptide comprising amino acids 1-120 of SEQ ID NO:33. The prior art does not disclose or

Application/Control Number: 08/585,895

Page 7

Art Unit: 1646

teach a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33. Specific claims to the embodiment of polynucleotide encoding a polypeptide consisting of amino acids 1-120 of SEQ ID NO:33 appear to be free of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christine Saoud, Ph.D., whose telephone number is (703) 305-7519. The examiner can normally be reached on Monday to Friday from 8AM to 3PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz, can be reached on (703) 308-4623. The fax phone number for this Group is (703) 308-0294.

Official papers filed by fax should be directed to (703) 308-4227. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

April 3, 2000

**CHRISTINE SAOUD
PATENT EXAMINER**

Christine Saoud

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.)
Serial No: 08/585,895)
Filed: January 12, 1996)
Title: Receptor Ligand)
Group Art Unit: 1646)
Examiner: Christine Saoud)

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned attorney of record in the above-identified application
hereby appoints as associate attorney(s):

Frank S. DiGiglio (Reg. No. 31,346)
Scully, Scott, Murphy & Presser
400 Garden City Plaza
Garden City, New York 11530
(516) 742-4343

to prosecute this application, to make alterations or amendments therein, and to
transact any and all business in the Patent and Trademark Office connected
therewith.

MARSHALL, OTOOLE, GERSTEIN,
MURRAY & BORUN



David A. Gass
Registration No. 38,153

June 22, 2000





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|-----------------|-------------|----------------------|---------------------|
| 08/585,895 | 01/12/96 | ALFREDO | 28113/33072 |

HM22/0629
FRANK S. DISIELLO
SCULLY SCOTT MURPHY & PRESSER
400 GARDEN CITY PLAZA
GARDEN CITY NY 11530

| EXAMINER |
|----------|
| SABUD, C |

| ART UNIT | PAPER NUMBER |
|----------|--------------|
| 1647 | 32 |

DATE MAILED: 06/29/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|--------------------|-------------|-----------------------|---------------------|
| 08/585,895 | | | |

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| EXAMINER |
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| ART UNIT | PAPER NUMBER |
|----------|--------------|

31

DATE MAILED:

INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Christine Saoud (3) DAVID GASS
(2) Gary Kunz (4) WILLIAM MERKEL
Date of Interview June 22, 2000 (5) FRANK J. DiGiglio

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description:

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 1, 3, 33, 18

Identification of prior art discussed: Hu et al. - of record in last office action.

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed claims w/ hybridization language as it relates to written description and enablement for prod. which binds Fil 4. Discussed host cell clms and product of mature (truncated) VEGF-C. Host cells which produce mature VEGF-C distinguish over the prior art of record.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM PTOL-413 (REV. 1-99)

Christine Saoud



FAU 1646 RECEIVED

AUG 15 2000

TECH CENTER 1600/2500

PATENT

#33
177

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|---------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1646 |
| Filed: January 12, 1996 |) | Examiner: Christine Saoud |

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

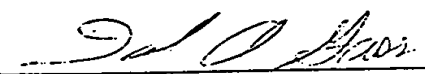
Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111;
2. Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo (unsigned); and
3. Check in the amount of \$91.00 in payment of fee for extension of time (\$55.00) and fee for extra claims (\$36.00).

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 4, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

08/11/2000 JADB01 00000011 08585895

01 FC:215

55.00 DP

RECEIVED

2011

RECEIVED

1. Small Entity Status

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.
 Has not been established.

| CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY) | | | | | | |
|---|----------------------------------|---------------------------------|--------------|---------|---------------------------|-----|
| | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
| | Claims Remaining After Amendment | Highest No. Previously Paid For | RATE | FEE | RATE | FEE |
| TOTAL | 44 | MINUS 40 = 4 | X 9 = | \$36.00 | X 18 = | \$ |
| INDEP. | 7 | MINUS 7 = 0 | X 39 = | \$ | X 78 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | + 130 = | \$ | + 260 = | \$ |
| Filing Fee: | | | | \$36.00 | OR | \$ |

2. Extension of Time

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | FEE FOR LARGE ENTITY | FEE FOR SMALL ENTITY |
|--------------------|----------------------|----------------------|
| One Month | \$110.00 | X \$55.00 |
| Two Months | \$380.00 | \$190.00 |
| Three Months | \$870.00 | \$435.00 |
| Four Months | \$1,360.00 | \$680.00 |
| Five Months | \$1,850.00 | \$925.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$ _____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

RECEIVED

AUG 15 2000

TECH CENTER 1600/2

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Petition is enclosed.

4. Deposit Account and Refund Authorization

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

August 4, 2000



RECEIVED
AUG 15 2000
TECH CENTER
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|---------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1646 |
| Filed: January 12, 1996 |) | Examiner: Christine Saoud |

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

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3. Check in the amount of \$91.00 in payment of fee for extension of time (\$55.00) and fee for extra claims (\$36.00).

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 4, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.
 Has not been established.

| CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY) | | | | | | |
|---|----------------------------------|---------------------------------|--------------|---------|---------------------------|-----|
| | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
| | Claims Remaining After Amendment | Highest No. Previously Paid For | RATE | FEE | RATE | FEE |
| TOTAL | 44 | MINUS 40 = 4 | X 9 = | \$36.00 | X 18 = | \$ |
| INDEP. | 7 | MINUS 7 = 0 | X 39 = | \$ | X 78 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | + 130 = | \$ | + 260 = | \$ |
| Filing Fee: | | | | \$36.00 | OR | \$ |

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | FEE FOR LARGE ENTITY | | FEE FOR SMALL ENTITY | |
|--------------------|----------------------|------------|----------------------|----------|
| One Month | | \$110.00 | X | \$55.00 |
| Two Months | | \$380.00 | | \$190.00 |
| Three Months | | \$870.00 | | \$435.00 |
| Four Months | | \$1,360.00 | | \$680.00 |
| Five Months | | \$1,850.00 | | \$925.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$55.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$ _____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

Extension Fee Due With This Request: \$55.00

3. Method of Payment of Fees

- ☒ Attached is a check in the amount of \$91.00
- ☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Petition is enclosed.

4. Deposit Account and Refund Authorization

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: _____

David A. Gass
Reg. No: 38,153

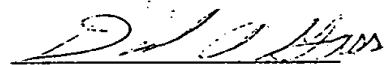
August 4, 2000



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|---|
| Applicant(s): Alitalo et al. |) | I hereby certify that this paper is being |
| Serial No: 08/585,895 |) | deposited with the United States Postal |
| Filed: January 12, 1996 |) | Service with sufficient postage as first class |
| Title: RECEPTOR LIGAND |) | mail, postage prepaid, in an envelope |
| |) | addressed to: Assistant Commissioner for |
| |) | Patents, Washington, D.C., 20231 on this |
| |) | date: |
| |) | |
| Group Art Unit: 1646 |) | Date: August 4, 2000 |
| Examiner: Christine Saoud |) | |
| |) |  |
| |) | David A. Gass |
| |) | Registration No. 38,153 |
| |) | Attorney for Applicants |
| |) | |

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an Office action mailed April 4, 2000, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 variously under 35 USC §§ 102(e) and 112, first paragraph. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This amendment is timely filed with a petition and fee for one month extension of time.

08/11/2000 JADD01 00000011 08585895

02 FC:203

36.00 DP

AMENDMENTS

In the claims:

Please cancel all pending claims and add new claims 45-79 as shown below:

~~44~~⁴⁶. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds to human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

~~46~~⁴⁷. A purified and isolated nucleic acid according to claim ~~45~~⁴⁶ wherein said polypeptide stimulates tyrosine phosphorylation of human Flt4.

~~47~~⁴⁸. A purified and isolated nucleic acid according to claim ~~46~~⁴⁷ wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

~~48~~⁴⁹. A purified and isolated nucleic acid according to claim ~~47~~⁴⁸ wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

~~49~~⁵⁰. A purified and isolated nucleic acid according to claim ~~48~~⁴⁹ wherein said polypeptide comprises approximately 120 amino acids.

~~50~~⁵¹. A purified and isolated nucleic acid according to claim ~~49~~⁵⁰ wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence identical to amino acids 2 through 18 set forth in SEQ ID NO: 13.

~~51~~⁵². A purified and isolated nucleic acid according to claim ~~50~~⁵¹ wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.

⁸
~~52.~~ A purified and isolated nucleic acid according to claim ~~48~~ wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

⁹
~~53.~~ A nucleic acid according to claim ~~48~~ wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes eight cysteines of SEQ ID NO: 33 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and excludes the carboxyl terminal portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

¹⁰
~~54.~~ A nucleic acid according to claim ~~53~~ wherein said continuous portion has amino acid 1 of SEQ ID NO: 33 as its amino terminus.

¹¹
~~55.~~ A nucleic acid according to claim ~~54~~ wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

¹²
~~56.~~ A vector comprising a nucleic acid according to claim ~~55~~, wherein said vector lacks a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

¹³
¹²
~~57.~~ A host cell transformed or transfected with a vector according to claim ~~56~~.

¹⁴
~~58.~~ A method for producing a polypeptide that binds to the extracellular domain of human Flt4, comprising the steps of:
growing a host cell according to claim ¹³ under conditions which permit expression by said host cell of a polypeptide that is encoded by said nucleic acid and that binds to the extracellular domain of human Flt4; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

¹⁵
~~55~~ A method according to claim ¹⁴~~56~~ wherein said host cell is a mammalian host cell that secretes said polypeptide and wherein said isolating step comprises isolating said polypeptide from said growth medium.

¹⁶
~~60~~ A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that binds human Flt4 receptor tyrosine kinase (Flt4), said polypeptide having an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

¹⁷
~~61~~ A purified and isolated nucleic acid according to claim ¹⁶~~60~~ wherein said encoded polypeptide stimulates tyrosine phosphorylation of human Flt4.

¹⁸
~~62~~ A purified and isolated nucleic acid according to claim ¹⁶~~60~~ wherein said nucleic acid lacks a nucleotide sequence that encodes the amino terminal portion of the amino acid sequence shown in SEQ ID NO: 33 that precedes residue 1.

¹⁹
~~63~~ An expression construct comprising the nucleic acid according to claim ¹⁸~~62~~ operatively linked to an expression control sequence, said expression construct lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125.

²⁰
~~64~~ A host cell transformed or transfected with the expression construct of claim ¹⁹~~63~~.

²¹
~~65~~ A method for producing a polypeptide that binds to the extracellular domain of human Flt4 and stimulates tyrosine phosphorylation of Flt4, comprising the steps of:

growing a host cell according to claim ~~64~~ under conditions which permit expression in said host cell of a polypeptide encoded by said nucleic acid and isolating said polypeptide from the host cell or the growth medium of the host cell, wherein said polypeptide binds to the extracellular domain of human Flt4 and stimulates phosphorylation of Flt4.

²²/₆₆ A host cell transformed or transfected with a polynucleotide, wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

²¹ (b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions and includes a domain encoded by the human nucleotide sequence that is defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

wherein said polypeptide binds to the extracellular domain of human Flt4 receptor tyrosine kinase.

²³/₆₇ A host cell according to claim ²²/₆₆ that expresses a naturally occurring human Flt4 ligand polypeptide encoded by said polynucleotide.

²⁴
~~68.~~ A host cell according to claim 66 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to sequence that encodes said polypeptide.

²⁵
~~69.~~ A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a contiguous portion of SEQ ID NO: 33 that is sufficient to bind to the extracellular domain of human Flt4 receptor tyrosine kinase (Flt4EC),

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B),

wherein said polypeptide lacks any portion of SEQ ID NO: 33 that precedes position 1 and lacks any portion of SEQ ID NO: 33 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P), and

²¹
wherein said polypeptide has a molecular weight of about 23 kD as assessed by SDS PAGE under reducing conditions and binds to Flt4EC.

²⁶
~~70.~~ A host cell according to claim ²⁵~~68~~ wherein said nucleotide sequence comprises nucleotides 37 to 1086 of the sequence shown in SEQ ID NO: 32.

²⁷
~~71.~~ A host cell according to claim ²⁵~~69~~ wherein said polynucleotide is a vector comprising an expression control sequence operatively linked to the nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 33.

²⁸
~~72.~~ A eukaryotic host cell according to claim ^{22 25}~~66~~ or ~~69~~ that secretes said polypeptide.

²⁹
~~73.~~ A host cell comprising the insert of plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses and secretes a polypeptide encoded by said insert,

wherein said secreted polypeptide has a molecular weight of about 23kD as assessed by SDS-PAGE under reducing conditions and binds to human Flt4 receptor tyrosine kinase and includes a domain defined by eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B).

30. A host cell transformed or transfected with a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that binds to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand containing a human nucleotide sequence that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

21 (b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses and secretes a polypeptide encoded by said polynucleotide, and

wherein said expressed and secreted polypeptide binds the extracellular domain of human Flt4 receptor tyrosine kinase and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

31 32. A method for producing a polypeptide that binds the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 25-31, 33, or 34 under conditions which permit expression by said host cell of said polypeptide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

³² 30. A method for producing a polypeptide that binds to the extracellular domain (EC) of human Flt4 receptor tyrosine kinase (Flt4), comprising steps of:

growing a host cell comprising a polynucleotide that comprises a nucleotide sequence that encodes the amino acid sequence set forth in SEQ ID NO:33, under conditions in which the host cell expresses and secretes a polypeptide encoded by the polynucleotide; and

isolating a polypeptide that binds Flt4 EC from the growth medium of the host cell, said polypeptide having a molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and having an amino acid sequence comprising a portion of SEQ ID NO:33 effective to bind Flt4 EC.

³² 31. A method according to claim ³² 30 wherein said polynucleotide comprises an expression vector that comprises a nucleotide sequence that encodes the amino acid set forth in SEQ ID NO:33.

³⁴ 32. A method according to claim ³² 30 wherein said host cell comprises a PC-3 prostatic adenocarcinoma cell (ATCC CRL1435).

³⁵ 33. A method according to claim ³² 30 wherein said polynucleotide comprises the insert of plasma pFLT4-L, deposited as ATCC Accession No. 97231.--

REMARKS

I. Prosecution History.

The application as filed contained 16 claims. In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In an Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In an amendment dated November 26, 1997, the Applicants canceled claims 6, 13, and 17; amended claims 1, 3-5, 7, 11, 18, and 20; and added new claims 26-38. In an amendment dated July 23, 1998, the Applicants amend claims 1, 3-5, 7, 18-19, 26-33, and

36-37; and add new claims 39-44. Thereafter, the Patent Office suspended prosecution of the application for approximately 18 months because "a reference relevant to the examination of this application may soon become available."

At the time of issuance of the outstanding Office action, claims 1, 3-5, 7, 11, and 18-44 were pending. In an interview on June 22, 2000, the Examiner requested submission of a renumbered claim set. Thus, the pending claims have been canceled and new claims 45-79 have been substituted therefor. A table correlating old and new claims is set forth for the Examiner's convenience.

| Current Claim | Corresponding Old Claim | Comments |
|---------------|-------------------------|----------|
| Claim 45. | Claim 18. | |
| Claim 46. | Claim 19. | |
| Claim 47. | Claim 20. | |
| Claim 48. | Claim 21. | |
| Claim 49. | Claim 22. | |
| Claim 50. | Claim 32. | |
| Claim 51. | Claim 11. | |
| Claim 52. | Claim 23. | |
| Claim 53. | Claim 30. | |
| Claim 54. | Claim 41. | |
| Claim 55. | Claim 31. | |
| Claim 56. | Claim 24. | |
| Claim 57. | Claim 25. | |
| Claim 58. | Claim 38. | |
| Claim 59. | Claim 39. | |
| Claim 60. | Claim 43. | |
| Claim 61. | Claim 19. | |
| Claim 62. | Claim 44. | |
| Claim 63. | Claim 34. | |
| Claim 64. | Claim 35. | |

| | | |
|-----------|-----------|--|
| Claim 65. | Claim 36. | |
| Claim 66. | Claim 1. | Additional limitations specifying human polynucleotide and 23 kD polypeptide |
| Claim 67. | Claim 26. | |
| Claim 68. | Claim 29. | |
| Claim 69. | Claim 3. | Additional limitation specifying 23 kD polypeptide |
| Claim 70. | Claim 4. | |
| Claim 71. | Claim 5. | |
| Claim 72. | Claim 40. | |
| Claim 73. | Claim 7. | |
| Claim 74. | Claim 42. | Additional limitation specifying human polypeptide |
| Claim 75. | Claim 37. | |

The suspension of this application for more than a year has been detrimental to the Applicants' continued commercial development of this technology, and the Applicants are quite interested in expeditious allowance, now that prosecution has resumed and the "relevant reference" has become available. The claim amendments herein are solely for the purpose of clarity and expediting allowance, and are unnecessary to overcome the Patent Office's rejections. The Applicants reserve the right to pursue the subject matter of claims as originally filed (or later introduced) in subsequent applications, such as continuing applications.

II. The Patent Office's rejection of claims 1, 37, and 42 under 35 U.S.C. §112, first paragraph, for lack of written descriptive support should be withdrawn.

In paragraph 6 of the Office action, the Patent Office rejected claims 1, 37, and 42 under 35 U.S.C. 112, first paragraph, alleging that these claims contain subject matter "which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention." Claim 37 was rejected due to its dependence from claim 1. New claims 66 and 74 are analogous to rejected claims 1 and 42.

Although the rejection spans approximately three pages, the Patent Office's principal objections appear to be that the breadth of the claims is such that the claims encompass polynucleotides from other species and polynucleotides which encode variant polypeptides so long as receptor binding activity is maintained. The Patent Office acknowledges the presence of Examples relating to a human Flt4 ligand, and there are no impediments to identifying other human molecules that may be allelic variants, for example. Solely to expedite allowance,¹ the Applicants have included a limitation in claims 66 and 74 to specify a human polynucleotide sequence. The Applicants' teachings of a human sequence, combined with their many additional teachings related to VEGF-C processing, Flt4 binding, Flt4 binding assays, hybridization techniques, and the like, conveys possession of the human genus recited in the claims to a person of ordinary skill. These amendments render moot the rejection for lack of written description, and the rejection should be withdrawn.

III. The Patent Office's rejection of claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e) should be withdrawn.

In paragraph 8 of the Office action, the Patent Office rejected claims 1, 3-5, 7, 11, and 18-44 under 35 U.S.C. §102(e), as allegedly being anticipated by Hu et al. (U.S. Pat. No. 5,935,820). The rejection specifically addressed only claims 11 and 18, although it contained some general reasoning apparently directed at the other claims:

Hu et al. disclose a polynucleotide, SEQ ID NO:1, which encodes a polypeptide, SEQ ID NO:2, which includes a domain defined by 8 cysteine residues of the VEGF family, and which is capable of binding to human Flt4 receptor tyrosine kinase. The instant claims indicate that the polypeptide lacks any domain that has one or more cysteine motifs of a Balbiani ring 3 protein, however, this limitation only further defines the processed protein and places no material limitations on the polynucleotide. Claim 11 further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide. Claim 18 is directed to a polynucleotide which lacks a

¹ The Applicants reserve the right to dispute the factual and legal premises upon which the rejection is based, and pursue claims of the original or greater scope in continuing applications.

The Applicants also observe that the Hu et al. '820 patent cited in paragraph 7 teaches only a single polynucleotide species yet purports to claim a genus using hybridization claim limitations. See, e.g., Hu et al. claims 41 and 50. The Examiner is requested to clarify the Patent Office's position as to when a single polynucleotide species provides a written description of a hybridization genus that satisfies §112, first paragraph.

portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a "polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" anticipates this claim.

(Office action at p.6.)

The Applicants respectfully traverse.

At the outset, the Applicants wish to clarify certain factual and legal issues raised by the above-quoted rejection. First, the rejection is factually incorrect in that Hu et al. neither discloses nor suggests that any polypeptide binds actually binds to Flt4. In fact, Hu et al. makes no mention of the Flt4 receptor whatsoever, and fails to identify any receptor for "VEGF2" whatsoever. Second, the Applicants object to the Patent Office's suggestion that the scope or wording of the claims of the Hu et al. patent have any relevance to whether Hu et al. is anticipatory under §102(e). The application that matured into the Hu et al. patent was filed on March 27, 1997, more than *two years after* the filing date of the present application, and *after the publication of a PCT application based on the present application* (See WO 97/05250, published February 13, 1997), and *after the publication of the present inventors own work in prominent scientific journals* that would have come to the attention of Hu et al.² Still more of the present inventor's publications were available to Hu et al. in 1997-1999, during prosecution of the Hu et al. application. (See, e.g., Joukov et al., "Proteolytic Processing regulates receptor specificity and activity of VEGF-C," *EMBO J.*, 16(13): 3898-3911 (1997).) The relevant inquiry under §102(e) is the inquiry of what was "described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent." This inquiry requires the Patent Office to ignore what was claimed in the Hu et al. patent, which may have been tainted by knowledge of the present invention, as explained above. The relevant inquiry must focus on what was *described* in those Hu et al. priority applications that have a filing date that could have

² See, e.g., Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2): 290-298 (1996); and Kukkk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V, Alitalo K., "VEGF-C receptor binding and pattern of expression with VEGFR-3 suggests a role in lymphatic vascular development," *Development*, 122(12): 3829-37 (1996).

preceded the invention date of the applicants.³ See, e.g., *In re Benno*, 226 USPQ 683, 686 (Fed. Cir. 1985) ("The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes....") The limited teachings of Hu et al. are discussed below.

The Applicants also dispute that the Patent Office's reasoning, even if correct, supports a legitimate anticipation rejection. Independent claims 1, 3, 7, and 42 (now claims 66, 69, 72, and 74) are all directed to host cells that have been transformed or transfected with a nucleic acid and that express an approximately 23 kD polypeptide encoded by the nucleic acid that has particular structural and functional characteristics, such as a particular size or sequence and/or the ability to bind the Flt4 receptor. The Hu et al. patent neither discloses nor suggests the recited polypeptides, or host cells that make such polypeptides, or the activities of the polypeptides. (There is no description in Hu et al. of a 23 kD Flt4 ligand polypeptide or of a host cell that produces such a polypeptide.)

Notwithstanding these claim limitations, the Patent Office rejected the claims. Under the Patent Office's analysis, "this limitation only further defines the processed protein and places no material limitations on the polynucleotide." The Patent Office has apparently ignored the fact that these particular claims are not directed to an isolated polynucleotide, but rather to a host cell that produces a polypeptide having certain characteristics. The Patent Office has failed to explain why a limitation on a novel and nonobvious protein produced by a host cell is insufficient to render novel a claim to the host cell that produces the protein. The Patent Office has apparently ignored the axiom that anticipation of a claim under §102 can be found only if the prior art discloses *every element* of the claim. See, e.g., *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986). An anticipation of a recombinant, protein-producing host cell claim does not exist merely because a polynucleotide has allegedly been described in the prior art.

The Patent Office rejected claim 18 (now claim 45), directed to a polynucleotide, on the basis that "Claim 18 is directed to a polynucleotide which lacks a portion of the nucleic acid sequence which encodes the cysteine motifs of a Balbiani ring 3 protein, but still encodes a polypeptide that is capable of binding to human Flt4 receptor

³ The Applicants reserve the right to dispute whether Hu et al. qualifies as a §102(e) reference, on the grounds that Hu et al. is not a patent granted on an application filed before the invention thereof by the applicant.

tyrosine kinase. This limitation appears to be inherently met by the embodiment of claim 1 of '820 in that the mature protein lacks this portion of the polypeptide, therefore, a 'polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2' anticipates this claim." This reasoning is based on an improper focus on what the cited patent *claimed*, rather than what it *described*. See *In re Benno, supra*. If one reads the Hu et al. patent to determine what Hu et al. actually *describes* as "a polynucleotide encoding a mature portion of a protein consisting of SEQ ID NO:2" one finds descriptions such as the following:

The polynucleotide of this invention . . . contains an open reading frame encoding a protein of about 350 amino acid residues of which approximately the first 24 amino acid residues are likely to be leader sequence such that the mature protein comprises 326 amino acids.

(Hu et al. at Col. 3, lines 56-63.)

Thus, Hu et al. describes a polynucleotide that encodes the "mature portion of a protein consisting of SEQ ID NO: 2" as a polynucleotide that comprises the final 326 codons of SEQ ID NO: 2.⁴ A study of the approximately 350 codon sequence in Hu et al. (Figures 1-2; SEQ ID NO: 2) shows that the mature protein of 326 amino acids includes the carboxy-terminal domain Balbiani Ring 3 Protein cysteine motifs. Because the "mature protein" described in Hu et al. includes the BR3P domain and falls outside the scope of claim 45, claim 45 is not anticipated. Claim 11 (now 51), which depends indirectly from claim 45, also is not anticipated.

In paragraph 9, the Patent Office acknowledged that certain subject matter was free of the prior art, and in paragraph 8, it suggested that claim 11 might have been an attempt to claim that allowable subject matter, except that its limitation "further defines the polypeptide as comprising amino acids 1 to 120 of SEQ ID NO:33, however, this limitation places no material limitations on the polynucleotide." Claim 11 (now claim 51) is patentable over the art because it depends from claim 45, as explained above. However, the Applicants wish to direct the Patent Office's attention to claim 60 (formerly 43), which claims a nucleic acid and contains the explicit limitation "said nucleic acid lacking a nucleotide sequence that encodes the carboxy-terminal portion of the amino acid sequence shown in SEQ ID NO: 33 beyond residue 125." The Applicants respectfully submit that claim 60 satisfies the Patent

⁴ The Applicants reserve the right to present evidence that the alleged signal peptide taught in Hu et al. does not operate as a signal peptide at all.

Office's own criteria for allowable subject matter in this case, and should not have been rejected at all.

For the foregoing reasons, the Hu et al. patents neither disclose nor suggest the claimed invention, and the rejections based on Hu et al. under 35 U.S.C. §102(e) should be withdrawn.

IV. Interview Follow-up

During the interview with Examiners Saoud and Kunz and the Applicants' attorneys, the Examiners acknowledged that host cells which produced a fully processed VEGF-C polypeptide of approximately 23 kD were novel and unobvious over the two Hu et al. patents of record, and raised the question of whether this result of the Applicants' was due uniquely to the host cell chosen. The Applicants have filed herewith a declaration to provide evidence that they have succeeded in producing a Flt4 ligand of approximately 23 kD in several other host cells. An executed version of the declaration will be submitted under separate cover.

V. Information Disclosure

On March 21, 2000, the Patent Office issued a third patent to Hu et al., U.S. patent No. 6,040,157. The '157 patent is a CIP that was filed in December, 1997, after many publications by the present applicants and after the present application was filed. The Applicants wish to draw the Examiner's attention to the '157 patent. For §102(e) purposes, the '157 patent is cumulative to the '540 and '820 patents of record. (To the extent it is not cumulative in disclosure, it is not citable as *prima facie* prior art, because the non-cumulative disclosure is not entitled a date that precedes the January 12, 1996, filing date of the present application.)

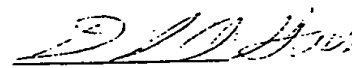
VI. Summary

The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: August 4, 2000


David A. Gass
Registration No. 38,153



RECEIVED

AUG 13 2000

1646
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.) Title: RECEPTOR LIGAND
Serial No: 08/585,895) Group Art Unit: 1646
Filed: January 12, 1996) Examiner: Christine Saoud

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an executed declaration of Kari Alitalo for entry into the file for the above-identified matter. An unsigned version of this declaration was previously filed, together with an amendment, on August 4, 2000. The Applicants believe that this declaration should be entered without petition or fee for additional extension of time, because a fully responsive amendment to the Office action of April 4, 2000, has already been filed. However, if extension of time is required, please consider this transmittal to be a request therefor, and charge an additional extension fee to deposit account No. 13-2855.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 10, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass



RECEIVED

AUG 18 2000

PATENT

TECH CENTER 1600/2500

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|---------------------------|
| Applicant(s): Alitalo et al. |) | Title: RECEPTOR LIGAND |
| Serial No: 08/585,895 |) | Group Art Unit: 1646 |
| Filed: January 12, 1996 |) | Examiner: Christine Saoud |

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith is an executed declaration of Kari Alitalo for entry into the file for the above-identified matter. An unsigned version of this declaration was previously filed, together with an amendment, on August 4, 2000. The Applicants believe that this declaration should be entered without petition or fee for additional extension of time, because a fully responsive amendment to the Office action of April 4, 2000, has already been filed. However, if extension of time is required, please consider this transmittal to be a request therefor, and charge an additional extension fee to deposit account No. 13-2855.

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David A. Gass



#34
1/21/00

PATENT
Attorney Docket No. 28967/33072
LUD 5453.1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo et al.

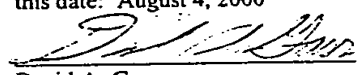
Serial No.: 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Group Art Unit: 1646

Examiner: SAOUD, Christine

) I hereby certify that this paper and the
) documents referred to as enclosed
) herewith are being deposited with the
) United States Postal Service as First Class
) Mail, postage prepaid, in an envelope
) addressed to: Assistant Commissioner
) for Patents, Washington, DC 20231, on
) this date: August 4, 2000
) 
) David A. Gass
) Reg. No.: 38,153
) Attorney for Applicants
)

Declaration Pursuant to 37 C.F.R. § 1.132 of Kari Alitalo

I, Dr. Kari Alitalo, declare and state as follows:

Introduction

1. I am a co-inventor of the subject matter of the above-identified patent application (hereinafter "the patent application"). I make this declaration to provide evidence to the Patent Office that may be relevant to the patentability of pending claims. Specifically, some of the pending claims in the application relate to recombinant host cells that produce a mature Flt4 receptor ligand polypeptide of approximately 23 kD (as assessed by SDS-PAGE under reducing conditions). This declaration is intended to provide evidence and confirmation that we have achieved expression of this mature form using a variety of host cells transformed/transfected with a full length cDNA encoding a 419 residue prepro-form of the ligand.

Evidence

I. Introduction

2. My co-inventor Dr. Joukov and I (together with others in my laboratory) have conducted substantial experiments to evaluate the proteolytic processing of human prepro-VEGF-C, a protein of 419 amino acids, into a mature form which has undergone substantial N-terminal and C-terminal processing. The results of many of the VEGF-C processing experiments are succinctly and accurately reported in our publication Joukov *et al.*, "Proteolytic processing regulates receptor specificity and activity of VEGF-C," *EMBO J.* 16(13): 3898-3911 (1997). I hereby reaffirm the accuracy of the data reported in that paper, which I incorporate by reference, and summarize only briefly in the next paragraphs of the introduction.

3. The VEGF-C gene encodes a mRNA for the synthesis of a prepro-protein F114 ligand precursor of 419 amino acids. (The complete 419 codon cDNA was deposited with the ATCC and is cross-referenced at pages 6, 28-29, and 39 of the patent application, and its sequence is deposited as SEQ ID NOs: 44 and 45 in the Sequence Listing.) The "pre-pro-protein" is processed to remove a signal peptide and two pro-peptides to produce a fully mature, most active form of VEGF-C. Initially, the "pre" part or signal sequence is cleaved off upon its translocation through the cellular membrane in the rough endoplasmic reticulum of the synthesizing cells. The rest of the polypeptide is then translocated across the cell membrane upon its continued elongation synthesis.

4. A proteolytic cleavage to cleave a C-terminal pro-peptide occurs preferentially between amino acid residues 227 and 228, separating the N-terminal and C-terminal halves (roughly) of the constituent polypeptides.¹ This polypeptide, once cleaved in the middle, is

¹ The C-terminal half contains cysteine residue repeat patterns reminiscent of the Balbiani Ring 3 Protein (BR3P). The N-terminal half contains a series of cysteine residues in a pattern shared with other members of VEGF/PDGF family.
Position 227 of the 419 codon sequence corresponds to position 125 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

only partially active relative to the fully mature, proteolytically processed VEGF-C that is created upon removal of the N-terminal pro-peptide from the N-terminal half.

5. Another cleavage of the VEGF-C protein precursor to remove an N-terminal pro-peptide occurs on the amino-terminal side of the domain that shares a cysteine motif in common with other members of the VEGF-PDGF family. In our experiments we have observed that this second pro-peptide cleavage occurs in at least two preferential peptide bonds: one is between amino acid residues 102 and 103,² and the other one between residues 111 and 112. These two alternative cleavages that remove the N-terminal pro-peptide produce fully processed forms of the Flt4 ligand which have a similar potency of high affinity binding to KDR/VEGFR-2, which is expressed in blood vascular endothelium and lymphatic endothelium and to Flt4/VEGFR-3, which is predominantly expressed in the lymphatic endothelium. The fully processed mature forms of VEGF-C that are most active have a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

II. Recombinant Host cells that produce a fully processed VEGF-C of about 23 kD

6. Several of our initial experiments were done using 293EBNA or 293 T-cells as host cell systems for the transfection of VEGF-C expression vectors. These cells were fairly efficient in the processing of the prepro-VEGF-C into the fully processed ~23 kD form. In the initial experiments described in our patent application, VEGF-C was eluted from

² Position 103 (Threonine) of the 419 codon sequence corresponds with position 1 of SEQ ID NO: 33, the sequence referred to in the claims of the patent application.

an Flt4-EC affinity matrix using pH 2.4, which seemed to enhance (but was not necessary for) the proteolytic processing into the mature form.³

7. We have also observed similar proteolytic processing in COS monkey cells and in the HT1080 human fibrosarcoma cells. Experimental details with COS and HT1080 cells are reported in Joukov *et al.* (1997), *supra*. The cells were grown in a conventional commercial media (Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum.⁴ The vector used for transformation of these cells was a pREP7 vector containing the 419 codon VEGF-C cDNA, essentially as described in the patent application. Processing to produce the ~ 23 kD form was observed in both COS and HT1080 cells, although it was significantly less efficient than in the 293 cells.

8. We have also used the MCF-7 breast carcinoma cell line to produce the ~23 kD mature form of VEGF-C. Unlike the 293 or COS cells, MCF-7 cells do not replicate the transfected plasmid. MCF-7 cells were transfected with pEBS7 expression vector⁵ containing a cDNA insert coding for human prepro-VEGF-C cDNA (419 codon form) or with an empty vector, and stable cell pools were selected. The transfected MCF-7 cells were grown in RPMI-1640 medium (a commercially available medium) containing 10% FCS and 150 µg/ml Hygromycin B. To study forms of VEGF-C produced, the cells were metabolically labeled,

³ Like all enzymatic reactions, the proteolytic cleavages in the VEGF-C polypeptide backbone occur in an enzyme- and substrate concentration- dependent manner, and can be influenced by factors such as pH, time, and protease inhibitors. Our data suggests that the enzymes that cleave the N-terminal pro-peptide from pro-VEGF-C are secreted from both VEGF-C-producing and from other cell types. We also have observed increased processing upon the depletion of the culture medium of fetal calf serum.

⁴ By way of comparison, Example 13 of the patent application describes culturing 293-EBNA cells in DMEM-0.2% BSA. As explained in the previous footnote, lower serum levels in the media appears to correlate with increased processing.

⁵ The pEBS7 expression vector was known in the literature at least as early as 1991, years before the priority date of the application. (See, e.g., Peterson, C. and Legerski, R., "High-frequency transfection of human repair-deficient cell lines by an Epstein Barr virus-based cDNA expression vector," *Gene*, 107(2): 279-284 (1991). We selected pEBS7 because this vector promotes high expression levels in MCF-7 cells. The vector contains a CMV promoter and hygromycin B and ampicillin resistance genes.

and after 96 hours, VEGF-C was bound to an Flt4 affinity substrate.⁶ The bound proteins were analyzed in 12.5% SDS-PAGE under reducing conditions. As can be seen from Figure A, the VEGF-C in the culture medium that bound to the soluble receptor Flt4-Ig affinity substrate consists of both the 29/31 kD and ~23 kD forms. Thus, expression of VEGF-C protein in these cells occurs in smaller quantities than other cells we have tested which replicate the transfected plasmid, yet we can recover both the 31/29 and ~23 kD forms of VEGF-C from the culture medium of these cells using the type of affinity matrix described in the patent application.

9. We also have achieved production of the ~23 kD form of VEGF-C using the baculovirus expression system in insect cells. In one set of the experiments, 3 million Sf-9 cells each were infected with baculoviral clones 32/1-32/5 and 34/1-34/5 expressing full length (419 codon) untagged hVEGF-C under the polyhedrin promoter.⁷ Seven days post-infection, the supernatant was harvested and the remaining cells lysed in 350 µl RIPA buffer. Ten microliters of supernatant of clone 32/1 and 5 µl lysate of clones 32/1 - 32/5 and 34/1 - 34/5 were subjected to 15% SDS PAGE, and VEGF-C specific bands were immunodetected after Western blotting using antiserum raised against VEGF-C peptide (residues 104-120, or

⁶ The affinity substrate that we used was Flt4(1-3)Fc, which comprises the three immunoglobulin-like regions of the Flt4 extracellular domain which have been shown to be responsible for ligand binding. Thus, this affinity matrix is the functional equivalent of the Flt4-EC affinity matrix described in the patent application.

⁷ The cell line Sf9 is a clonal isolate of Sf21 cells, which are derived from ovary cells of the fall army worm, *Spodoptera frugiperda*. The cell line was maintained as adherent culture at 27°C in TMN-FH media completed with fetal bovine serum to a final concentration of 10%. In addition, 100 mg/ml streptomycin and 10 units/ml penicillin were used to minimize the risk of bacterial contamination. The cells were cultured using routine and standard procedures for these experiments. (See, e.g., O'Reilly et al., *Baculovirus Expression Vectors: a laboratory manual*. W.H. Freeman and Company. New York, 1992: pp. 109-122).

For virus production and amplification, 5 baculoviral clones (1-5) were purified from two transfection supernatants (32 and 34), that were obtained using the FASTBAC system (GIBCO/Life Technologies) according to the instructions of the manufacturer. Transfections 32 and 34 were performed using two bacmid DNA preparations from independently obtained clones using shuttle vector pFB1-hVEGF-C-FL. Stock virus was obtained by two rounds of amplification after plaque purification. For the first amplification, 2.5 Mio. Sf-9 cells were inoculated with the whole purified plaque and incubated for 5 days. For the second amplification 8 Mio. Sf-9 cells were inoculated with 1/40 of the total virus obtained in the first amplification step and incubated for 5 days.

2-18 of the mature form). The results, depicted in Figure B, show clearly that the major form of VEGF-C in the lysates of 7 day p.i. cells is the 21/23 kD form. Uncleaved (prominent band), 29/31 kDa (prominent band), and ~23 kDa forms (weaker band) were present in the supernatant. These experiments show that insect cells also cleave the VEGF-C protein to the ~23 kD mature form and that this insect cell expression system can provide a source for large-scale production of the ~23 kD form of the protein.

10. In another series of experiments, we transfected the MeWo cell line,⁸ established from a lymph node metastasis of a nodular malignant melanoma, to constitutively overexpress a prepro-VEGF-C cDNA (419 codon form)⁹. Ordinary commercial media was employed for these experiments also (RPMI 1640 medium with 5% fetal bovine serum (FBS), purchased from Gibco BRL, Grand Island, NY). As determined by Northern analysis, the parental MeWo cell line and three vector-transfected control clones (MeWo/control) did not express any detectable amounts of VEGF-C mRNA *in vitro* or *in vivo*. Three VEGF-C transfected cell clones (MeWo/VEGF-C) expressed high levels of VEGF-C mRNA in culture, as well as in tumors (when introduced into mice) that reached the size of ~1200 mm³. Western blot analyses using antibodies raised against a VEGF-C peptide confirmed that high VEGF-C mRNA levels correlated with high amounts of VEGF-C protein expression. We

⁸ The human malignant melanoma cell line MeWo (Sordat, B.C. M., Y. Ueyama, and J. Fogh. 1982. Metastases of tumor xenografts in the nude mouse. In *The nude mouse in experimental and clinical research*. J. Fogh, and B.C. Giovanella, editors. Academic Press, New York. 95-147; Kerbel, R.S., M.S. Man, and D. Dexter. 1984. A model of human cancer metastasis: extensive spontaneous and artificial metastasis of a human pigmented melanoma and derived variant sublines in nude mice. *J Natl Cancer Inst.* 72:93-108), kindly provided by Dr. Robert S. Kerbel (Sunnybrook Health Science Centre, Toronto, Canada)

⁹ A 1997 bp full-length (419 codon) human VEGF-C cDNA (GenBank accession number X94216) was cloned into a pcDNA3.1/Zeo expression vector (Invitrogen, San Diego, CA) which contains a CMV-enhancer-promoter and a Zeocin selection cassette. The sequence and the orientation of the VEGF-C gene in the construct were verified by restriction mapping and by direct sequencing using the Sanger dideoxy method. Subconfluent cell cultures were transfected either with pcDNA3.1/Zeo vector containing the full-length human VEGF-C cDNA in sense orientation or with the vector alone using the Superfect transfection reagent (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. Forty-eight hours after transfection, cells were split 1:5 into their full growth medium containing 50 mg/ml Zeocin (Invitrogen) to select transfectants. Stably transfected cell clones were individually expanded and analyzed for VEGF-C mRNA expression and protein secretion.

detected a strong band of approximately 60 kDa in cell lysates of VEGF-C transfectants, corresponding to VEGF-C precursor, and only trace amounts in control cells. Large amounts of the secreted 31 kDa form were observed in culture supernatants of VEGF-C transfected clones, whereas the secreted protein was not detectable in supernatants of control cells. The mature ~23 kDa VEGF-C form was detected in tumor lysates.

11. The foregoing experiments demonstrate that we were able to recombinantly express the ~23 kD mature form of the Flt4 ligand VEGF-C in a variety of human cell lines transfected with a 419 codon prepro-VEGF-C cDNA, as well as in a Cos monkey cell line and an insect cell line.

Certification

12. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Kari Alitalo

Date: _____

Figure A

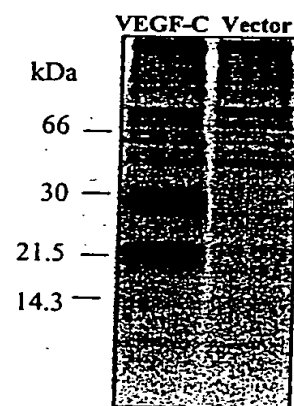
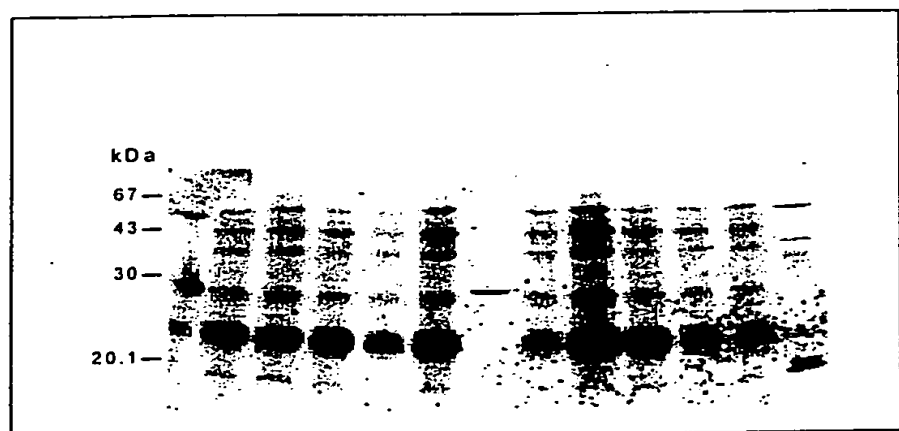


Figure B



#317/G
DM
2/24
PATENT

Attorney Docket No.: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|---|
| Applicant(s): Alitalo et al. |) | I hereby certify that this paper is being |
| Serial No: 08/585,895 |) | deposited with the United States Postal |
| Filed: January 12, 1996 |) | Service, in an envelope addressed to the: |
| Title: RECEPTOR LIGAND |) | Commissioner for Patents, Box Issue |
| Allowed: October 24, 2000 |) | Fee, Washington, D.C. 20231, utilizing |
| Batch No.: U18 |) | the "Express Mail Post Office" under |
| Group Art Unit: 1647 |) | Mailing Label No. EL566464161US on |
| Examiner: C. Saoud |) | this date: |
| |) | January 24, 2001 |



Suzanne A. Maguigad
Suzanne A. Maguigad

AMENDMENT AFTER ALLOWANCE PURSUANT 37 C.F.R. § 1.312

Commissioner for Patents
Box Issue Fee
Washington, D.C. 20231

Dear Sir:

Please amend this application as follows:

AMENDMENTS

In the Specification:

At page 1, line 3, after "August 1, 1995.", please delete the following priority claim, which was introduced by way of Amendment filed July 23, 1998:

"This application is also a continuation-in-part of U.S. Patent Application Serial No.

08/340,011, filed November 14, 1994, now U.S. Patent No. 5,776,755."

At page 8, line 7, please delete "Figure 2 schematically depicts" and insert --
Figures 2A and 2B schematically depict--.

At page 8, line 29, please delete "Figure 9B shows" and insert --Figures 9B-D
show--.

At page 8, line 30, please delete "Figures 10A-10B" which was introduced by
way of Amendment filed November 26, 1997 and insert --Figures 10A-D--.

At page 14, line 32, please delete "Figure 2" and insert --Figures 2A and 2B--.

At page 27, line 30, please delete "Figure 9B" and insert --Figures 9B through
9D--.

At page 28, line 1, please delete "Figure 10" and insert --Figures 10A through
10D--.

At page 28, line 6, please delete "Fig. 9B" and insert --Figures 9B through
9D--.

At page 29, line 13, please delete "Fig. 10" and insert --Figures 10B and
10C--.

REMARKS

Applicants request entry of the foregoing amendments, which relate solely to
formal matters. These amendments are being presented prior to or concurrently with payment
of the issue fee as required by Rule 312. The amendments do not affect the scope or content of
the allowed claims. The Patent Office is authorized to charge any fee associated with this
amendment to Deposit Account No. 13-2855.

The amendment to page 1 amounts to a cancellation of a priority claim to an

application that was filed in November, 1994. The Applicants continue to maintain their priority claim to U.S.S.N. 08/510,133, filed August 1, 1995, as stated in the application as originally filed. The sole purpose behind cancellation of the 1994 priority claim is to maximize patent term of the eventual patent, because it is the Applicants' understanding of current law that the term of this patent will be measured from the earliest claimed priority date. The priority claim cancellation is not intended as an admission of whether or not the claimed invention would be entitled to priority, if the priority claim to the November, 1994 application were maintained. The Applicants reserve the right to maintain the same priority claim for subject matter that may be pursued in related applications, such as continuations, continuations-in-part, divisional applications, reissue applications, or the like. It is the Applicants' understanding from prosecution that the subject matter of the allowed claims has been deemed patentably distinct from any subject matter disclosed in art of record, including subject matter disclosed in U.S. patent issued to Human Genome Sciences (Hu et al., U.S. Patent No. 5,935,820) that was considered by the Examiner. (This patent was cited by the Examiner as a reference under §102(e) and distinguished by the Applicants. See Amendment dated August 4, 2000, at pages 11-15.) Thus, the presence or absence of the priority claim raises no patentability issues.¹

The remaining amendments to the specification merely conform the specification to the formal drawings submitted concurrently herewith. Figures 2, 5, 9 and 10 were prepared

¹ The November, 1994 patent application has issued as U.S. Patent No. 5,776,755. The '755 patent is not prior art under §102(e) because, to the extent the '755 patent discloses or suggests the present invention, the relevant disclosure is a disclosure of the present inventors' own work. Because the relevant portions of the '755 patent constitute the inventor's own work, the relevant filing date of the '755 patent was not "before the invention thereof by the applicant" as required by §102(e). (It is impossible to disclose the inventors' own work before the inventors invented it.)

on multiple sheets and/or renumbered in order to comply with the Draftsman's requirements. The specification has been amended to reflect the fact that these figures will be multiple pages in the issued patent.

These amendment add no new matter and do not raise any new patentability issues that would require any substantive examination by the Examiner.

In view of the foregoing, the applicant respectfully requests the granting of the amendment after allowance.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,
MURRAY & BORUN

By: 

David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606

January 24, 2001

Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☒ Small entity status has been established and is still effective.
☐ Has not been established.

2. Deposit Account and Refund Authorization


- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:


David A. Gass
Reg. No: 38,153



Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------|---|-----------------------------|
| Applicants: Alitalo et al. |) | Allowed: October 24, 2000 |
| |) | Batch No.: U18 |
| Serial No: 08/585,895 |) | Application No.: 08/585,895 |
| |) | |
| Filed: January 12, 1996 |) | Title: RECEPTOR LIGAND |
| |) | |
| |) | Group Art Unit: 1647 |
| |) | |
| |) | Examiner: C. Saoud |

TRANSMITTAL LETTER

Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following for entry in the above-identified case:

1. Amendment After Allowance;
2. Thirty sheets of formal drawings (Figs. 1, 2A-2B, 3-4, 5A-5C, 6-8, 9A-9D, 10A-10D, 11-12, 13A-13B, 14A-14B, 15A-15B, 16A-16B, 17-18); and
3. Request for correction of Drawing with sketch showing proposed change to Figure.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper is being deposited with the United States Postal Service, in an envelope addressed to the: Commissioner for Patents, Box Issue Fee, Washington, D.C. 20231, utilizing the "Express Mail Post Office" under Mailing Label No. EL566464161US on January 24, 2001.

Suzanne A. Maguigad
Suzanne A. Maguigad

Allowed: October 24, 2000
Batch No.: U18
Application No.: 08/585,895

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☒ Small entity status has been established and is still effective.
- ☐ Has not been established.

2. Deposit Account and Refund Authorization

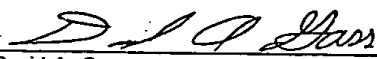
- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.
- ☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:

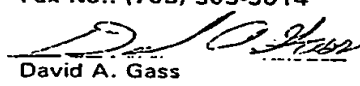

David A. Gass
Reg. No: 38,153

Nov. 2 2000 3:40PM MARSHALL, O'TOOLE

No. 5291 P. 2/2
From: 0819

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|------------------------------|---|---|
| Applicant(s): Alitalo et al. |) | I hereby certify that this paper is |
| Serial No: 08/585,895 |) | being sent via facsimile to: |
| Filed: January 12, 1996 |) | Commissioner for Patents, |
| Title: Receptor Ligand |) | Washington, D.C., 20231 on this |
| Group Art Unit: 1646 |) | date: Date: November 2, 2000. |
| Examiner: Christine Saoud |) | Fax No.: (703) 305-3014 |
| |) |  |
| |) | David A. Gass |
| |) | Registration No. 38,153 |
| |) | Attorney for Applicants |

Commissioner for Patents
Washington, D.C. 20231

CHANGE OF ADDRESS

Sir:

The undersigned is an attorney of record in this case. Please mail all correspondence in this case to the undersigned at the address below :

David A. Gass
Marshall, O'Toole, Gerstein, Murray & Borun
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402

The attorney's phone number is (312) 474-6300.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

Dated: November 2, 2000


David A. Gass
Registration No. 38,153

OK to Enter



Issue Date: October 24, 2000
Issue Batch No.: U18
Application No.: 08/585,895

PATENT
Attorney Docket No.: 28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Allowed: October 24, 2000

Batch No.: U18

Group Art Unit: 1647

Examiner: C. Saoud

) I hereby certify that this paper is being
) deposited with the United States Postal
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) Label No. EL566464161US on this date:

) January 24, 2001

) *Suzarah A. Maguigad*
) Suzarah A. Maguigad

REQUEST FOR APPROVAL OF DRAWING CHANGES

Commissioner for Patents
Washington, D.C. 20231
Attn: Official Draftsperson

Dear Sir:

AMENDMENT

Applicants hereby request approval of the drawing changes as shown in red ink on the attached copy of the informal drawing (FIG. 9B) for the above-identified application. Support for the requested change may be found throughout the specification as originally filed as explained below. No new matter has been added. The requested change is embodied in the formal drawings filed herewith. The Patent Office is authorized to charge any fee required in connection with the filing of this request to Deposit Account 13-2855.

#1
Pg
3/5

REMARKS

FIG. 9B illustrates the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA, in which the cleavage site for the putative signal peptide is indicated with a shaded triangle, as disclosed in the specification at page 8, lines 29-31. The drawing change is being made solely to correct the location of the shaded triangle which indicates the cleavage site demarking a mature VEGF-C protein. Particularly, the shaded triangle should be positioned between "Arg" and "Thr" and not "Ser and Arg". The position of the shaded triangle indicates the start of the designation of the portions of SEQ ID NO: 33 which correspond to the "mature" forms of VEGF-C. The change finds support as originally filed because the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27 to page 26, line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ ID NO: 25 that begin ACAGAAGAGACT...). Similar changes to the numbering of residues in the Sequence listing were made in an Amendment filed by the Applicants on November 26, 1997, and were approved by the Examiner. The changes made herein are consistent with what was earlier done the prosecution of this application.

In view of the foregoing, it is submitted that the change to FIG. 9B does not introduce new matter into the disclosure of the application or to the drawings. Accordingly, applicants request approval of the above drawing change.

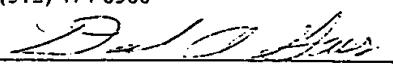
Corrected formal drawings will be provided for the above-identified
application.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

January 24, 2001

By:


David A. Gass
Registration No. 38,153
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606

MetThrValLeuTyrProGluTyr
 CAGCACTACCCCTCTCTCTCCAGCTGACATCACTCATGCTGACTCTACCCAGATAT
 10 30 50
 TrpLysMetTyrTyrCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
 TCGAAATGTATAGTGTACCTAAGGAAGAGGCTGGCAACATAACAGACAGAGCC
 70 90 110
 AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu
 AACCTCACTCTACGACAGACAGACTATAAATTGCTCCAGCATTATAATACACAG
 130 150 170
 IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
 ATCTTCAAAAGTATTGATAATCAGTGCAGAAAGACTCAATCCATCCACCCAGCTGT
 190 210 230
 IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
 ATAGATCTGCGGAGCACTTTCGAGTCCGACAAACCTTCTTAAACCTCCATGTGT
 250 270 290
 SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
 TCCCTTACACATCTCCCGCTTCTGCAATAGTACAGCGCTCCATCCATCAACACAGC
 310 330 350
 ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
 ACGAGCTACCTCAGCAGAGCTTATTGAAATTACAGTCCCTCTCTCAAGCCCCAAA
 370 390 410
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
 CCACTAACAATCTATTGCAATCAGACTTCTCCGATCCATGTCTAACTCCATGT
 430 450 470
 TyrArgGlnValHisSerIleIleArgSerLeuProAlaThrLeuProGlnCysGln
 TACAGACAGTTTATTCATTATTACAGCTTCCCTCCACACACTTCCAGTGTCTAC
 490 510 530
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
 CGAGCAACAGACCTCCCCCAATACATCTCGATATCAATCTCCAGATCCCTG
 550 570 590
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspSerThrAspGlyPheHis
 CCTCAGCAAGATTTTATCTTTCTCTGGATCTCCAGATCACTCAACACATCCATCCAT
 610 630 650
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
 CACATCTGTGACCAACAGAGAGCTCGATGACAGACCTCTCAGTGTCTCTCCAGAGC
 670 690 710
 GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
 GGGCTTCCGCTCCAGCTGTGACCCCAACAGAACTACAGACAACTCATGCCAGTGT
 730 750 770
 ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
 CTCTGTAAACAAACTCTTCCCAATGTGCGCCCAACCGCAATTGATCAAAAC
 790 810 830
 ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
 ACATGCCAGTGTGTATGTAAAGAACCTCCCCAGAAATCAACCCCTAAATCTCCGAAA
 850 870 890
 CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
 TGTGCTGTCAATCTACAGAACTCCACAGAAATGCTGTGTAAGGAAGAACTTCCAC
 910 930 950
 HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
 CACCAACATCCAGCTGTACAGACGCCATGTACGAACCCCCAGAGCTGTGTGACCA
 970 990 1010
 GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
 GCAATTTTATATCTCAAGACTGTCTCTCTGTCTCCCTTCATATTGGAAGACCAAA
 1030 1050 1070
 MetSerEnd
 ATGAGCTAAGATTGTACTGTTTCCAGTTTCATGATTTTCTATTATGAAAAGTGTGTG
 1090 1110 1130

FIG. 9B

APPROVED:
 DRAFTED:
 FIG.
 SUBCLASS

1/30

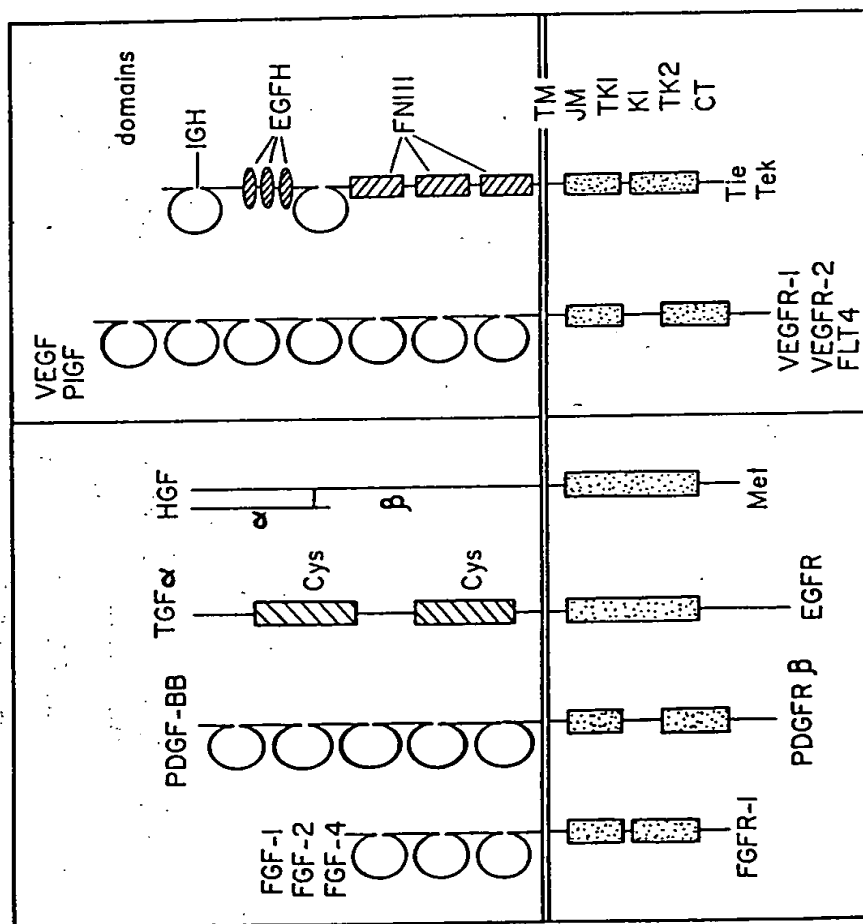
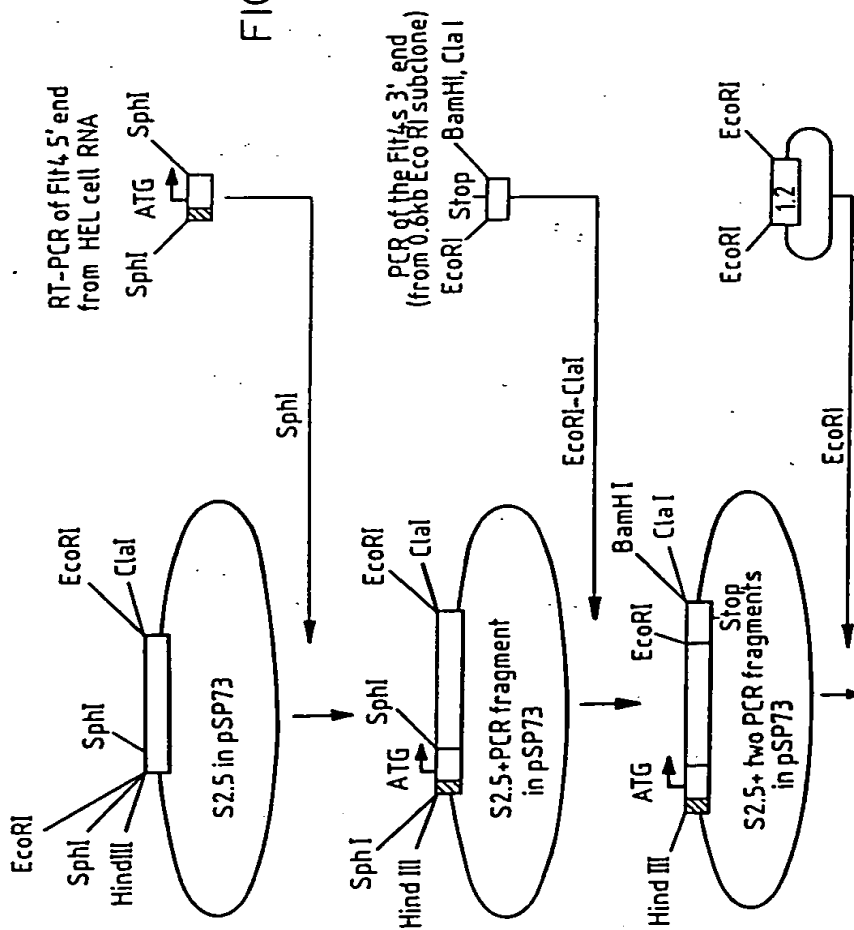


FIGURE 1

FIGURE 2A

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UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
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FROM : Ray M. Rayford, Manager
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SUBJECT: Receipt of Papers and Fees File Under 37 CFR 1.10 By
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The filing date of _____ is the correct date. The date on the Express Mail label under 37 CFR 1.10 is _____. On that date the PTO was closed all day due to _____ adverse weather conditions (authorized by Office of Personnel Management) or a _____ normally scheduled Federal holiday within the District of Columbia. In accordance with 37 CFR 1.6 the papers have been stamped with the next succeeding day which is not a Saturday, Sunday or Federal holiday within the District of Columbia. The provision of 35 U.S.C. 21 (b) apply.

The papers were not stamped with the date on the certificate of mailing by Express Mail because the date on the certificate does not coincide with the date of deposit on the Express Mail label which the PTO takes as evidence of when the package was mailed.

Date on certificate of mailing by Express Mail is 1-12-96

Date on Express Mail label is 1-12-96

Date of receipt in PTO is 1-16-96

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The papers are not entitled to the benefits of 37 CFR 1.10 because:

You are unable to receive the date on your
mail certificate or mailing label because of
the inclement weather conditions therefore you will
receive the date the PTO receive it which is 1-16-96

SIGNED: Julia H. Woodbridge

DATE : 1-19-96

PAGE: 1

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION US/08/585,895

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Original Text

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208 (B) TYPE: nucleic acid
209 (C) STRANDEDNESS: single
210 (D) TOPOLOGY: linear

211
212 (ii) MOLECULE TYPE: DNA (genomic)

213
214 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

215
216 GTTGCCTGTG ATGTGCACCA

20

217
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230 1 5 10 15

231
232 Leu Lys
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247 GCAGARGARA CNATHAA

17

248
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252 (B) TYPE: amino acid
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256 (ii) MOLECULE TYPE: peptide
257
258

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156 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
157 20 25 30
158
159 His Arg Gln Glu Ser Gly Phe Arg
160 35 40
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198 (ii) MOLECULE TYPE: DNA (genomic)
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202 CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC 34
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204 (2) INFORMATION FOR SEQ ID NO:12:
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119 (D) TOPOLOGY: linear
120
121 (ii) MOLECULE TYPE: DNA (genomic)
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133 (D) TOPOLOGY: linear
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135 (ii) MOLECULE TYPE: DNA (genomic)
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146 (C) STRANDEDNESS: single
147 (D) TOPOLOGY: linear
148
149 (ii) MOLECULE TYPE: protein
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151 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
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33

17

34

PAGE: 2

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

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TIME: 14:24:20

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49      (ii) MOLECULE TYPE: DNA (genomic)
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51      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
52
53      TGTCTCGCT GTCCTTGTCT
54
55      (2) INFORMATION FOR SEQ ID NO:2:
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57      (i) SEQUENCE CHARACTERISTICS:
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59          (B) TYPE: nucleic acid
60          (C) STRANDEDNESS: single
61          (D) TOPOLOGY: linear
62
63      (ii) MOLECULE TYPE: DNA (genomic)
64
65      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
66
67      ACATGCATGC CACCATGCAG CGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
68
69      GACTCCTGGA
70
71      (2) INFORMATION FOR SEQ ID NO:3:
72
73      (i) SEQUENCE CHARACTERISTICS:
74          (A) LENGTH: 24 base pairs
75          (B) TYPE: nucleic acid
76          (C) STRANDEDNESS: single
77          (D) TOPOLOGY: linear
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79      (ii) MOLECULE TYPE: DNA (genomic)
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81      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
82
83      ACATGCATGC CCCGCCGGTC ATCC
84
85      (2) INFORMATION FOR SEQ ID NO:4:
86
87      (i) SEQUENCE CHARACTERISTICS:
88          (A) LENGTH: 22 base pairs
89          (B) TYPE: nucleic acid
90          (C) STRANDEDNESS: single
91          (D) TOPOLOGY: linear
92
93      (ii) MOLECULE TYPE: DNA (genomic)
94
95      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
96
97      CGGAATTCCC CATGACCCCA AC
98
99      (2) INFORMATION FOR SEQ ID NO:5:
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TEAM 7

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895

DATE: 04/11/96
TIME: 14:24:17

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This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

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3 (1) General Information:
4
5 (i) APPLICANT: Alitalo, Kari
6 Joukov, Vladimir
7
8 (ii) TITLE OF INVENTION: Receptor Ligand
9
10 (iii) NUMBER OF SEQUENCES: 35
11
12 (iv) CORRESPONDENCE ADDRESS:
13 (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
14 (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
15 (C) CITY: Chicago
16 (D) STATE: Illinois
17 (E) COUNTRY: United States of America
18 (F) ZIP: 60606-6402
19
20 (v) COMPUTER READABLE FORM:
21 (A) MEDIUM TYPE: Floppy disk
22 (B) COMPUTER: IBM PC compatible
23 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
24 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
25
26 (vi) CURRENT APPLICATION DATA:
27 (A) APPLICATION NUMBER:
28 (B) FILING DATE:
29 (C) CLASSIFICATION:
30
31 (vii) ATTORNEY/AGENT INFORMATION:
32 (A) NAME: Gass, David A.
33 (B) REGISTRATION NUMBER: 38,153
34 (C) REFERENCE/DOCKET NUMBER: 28113/33072
35
36 (ix) TELECOMMUNICATION INFORMATION:
37 (A) TELEPHONE: 312/474-6300
38 (B) TELEFAX: 312/474-0448
39 (C) TELEX: 25-3856
40
41 (2) INFORMATION FOR SEQ ID NO:1:
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43 (i) SEQUENCE CHARACTERISTICS:
44 (A) LENGTH: 20 base pairs
45 (B) TYPE: nucleic acid
46 (C) STRANDEDNESS: single

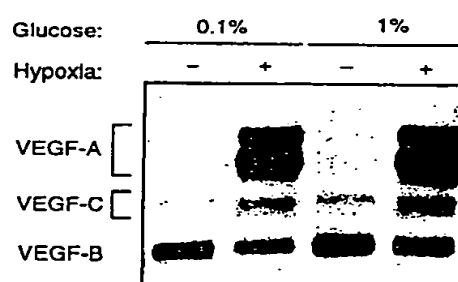
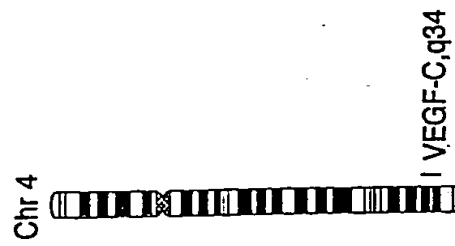


FIG. 18

FIG. 17



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FIG. 15B

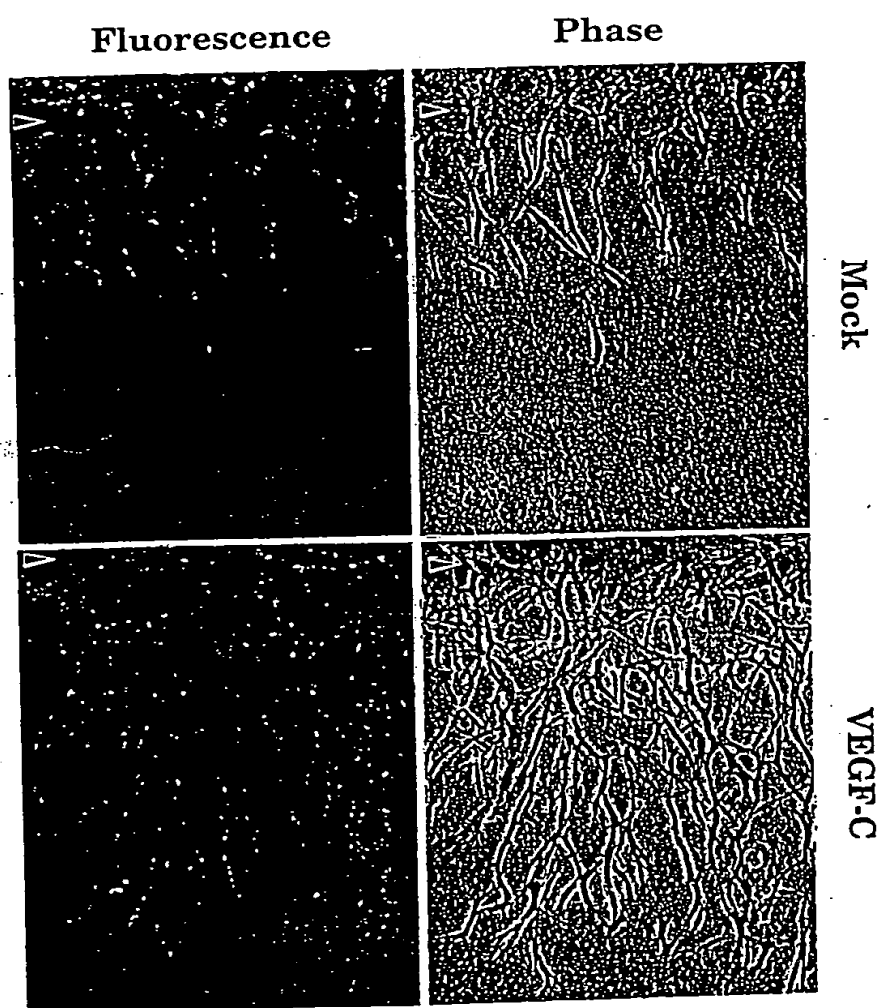


FIG. 15A

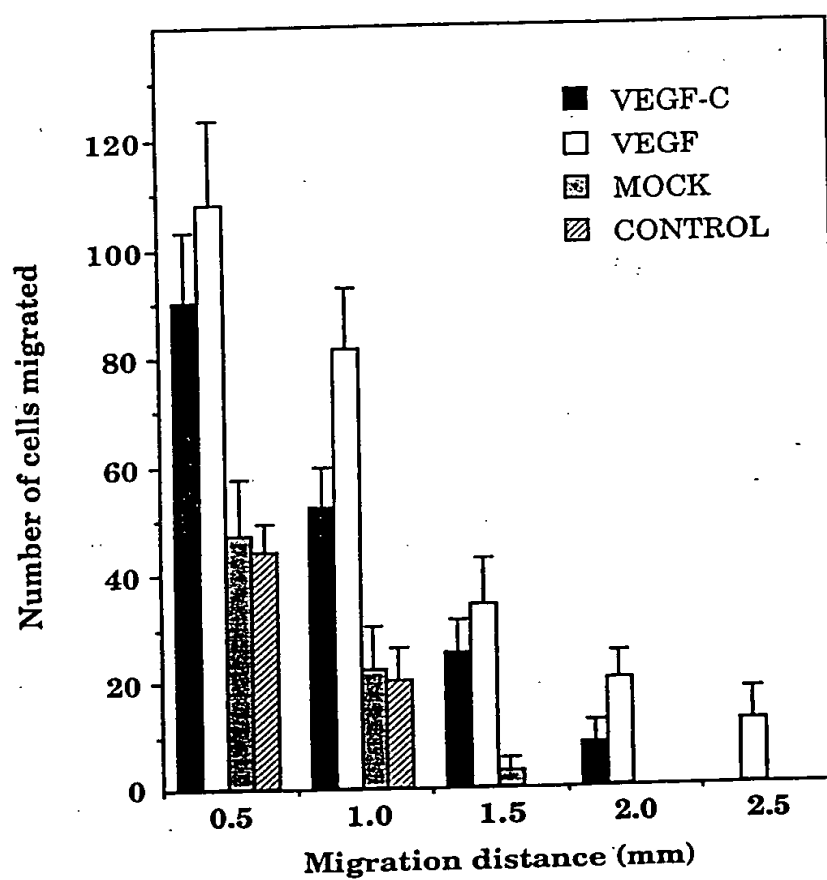
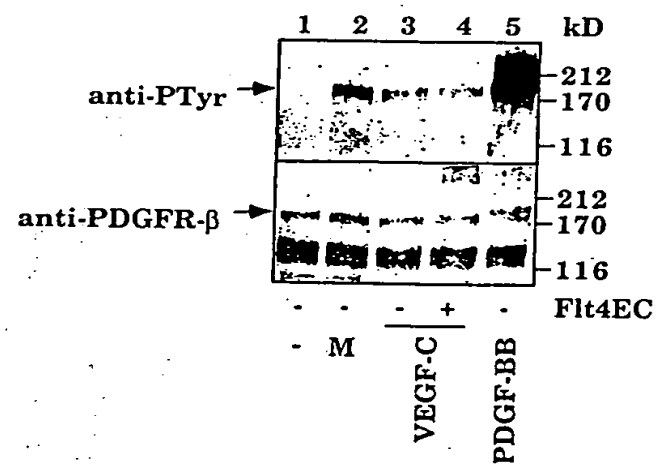


FIG. 14B



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FIG. 14A

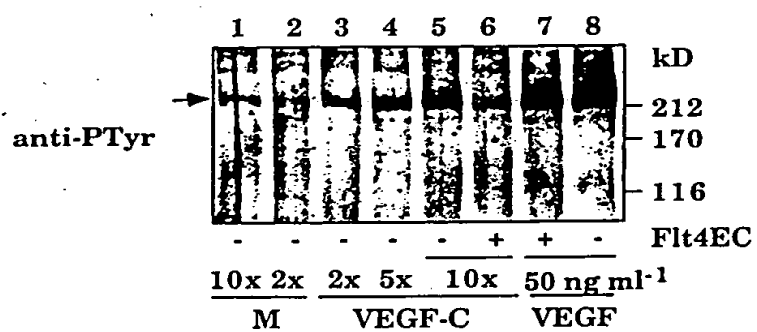
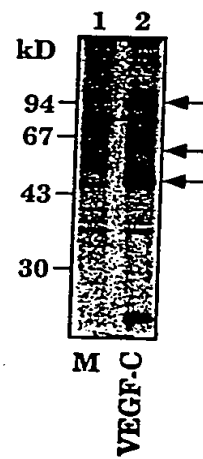


FIG. 13B



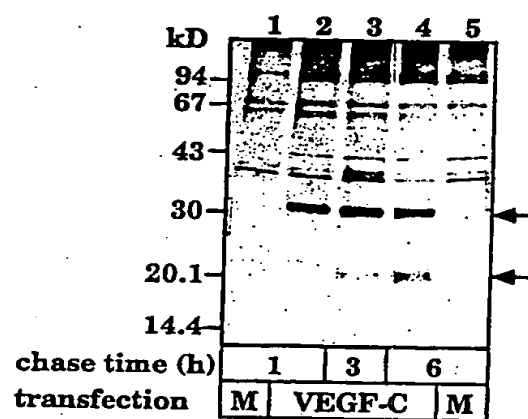


FIG. 13A

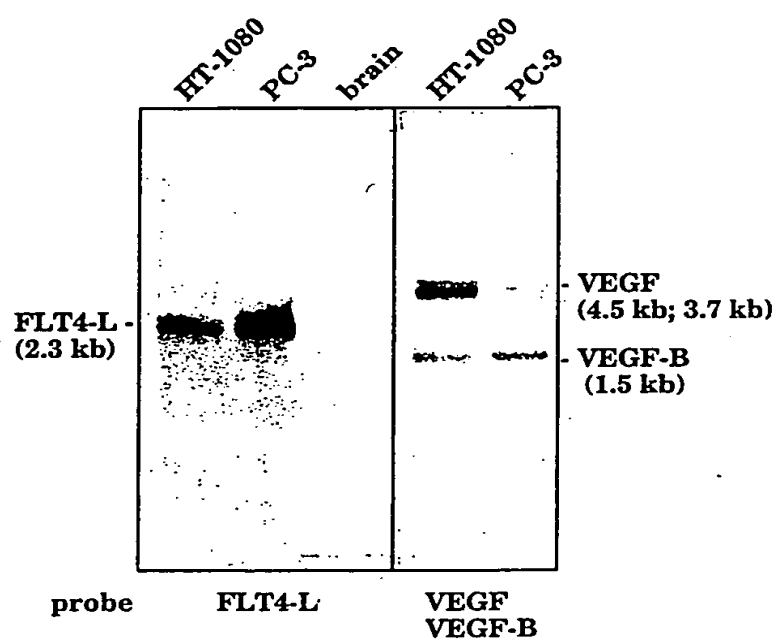


FIGURE 12

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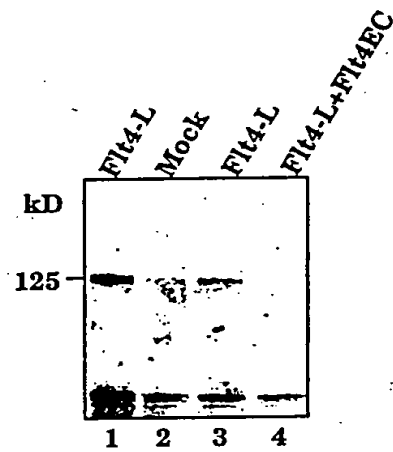


FIGURE 11

| | | | | | |
|---------|-------------|-------------|------------|------------|------------|
| | 251 | | | | 300 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | CGPCSEERRKH | LFVQDPQTCK | CSCKNIDSRC | KARQLELNER | TCRCDKPRR. |
| VEGF189 | CGPCSEERRKH | LFVQDPQTCK | CSCKNIDSRC | KARQLELNER | TCRCDKPRR. |
| VEGF206 | CGPCSEERRKH | LFVQDPQTCK | CSCKNIDSRC | KARQLELNER | TCRCDKPRR. |
| Flt4-L | FHDICGPNKE | LDEETCQCVC | RAGLAPASCG | PHKELDRNSC | QCVCCKNLFP |
| | 301 | | | | 350 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | | | | | |
| VEGF189 | | | | | |
| VEGF206 | | | | | |
| Flt4-L | SQCGANREFD | ENTCQCVCCKR | TCPRNQPLNP | GKCAECTES | PQRCLLKCKK |
| | 351 | | | | 394 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | | | | | |
| VEGF189 | | | | | |
| VEGF206 | | | | | |
| Flt4-L | FHHQTCSCYR | RPCTNRQKAC | EPGFSYSEEV | CRCVPSYWKR | PQMS |

FIG. 10 B

1 52

PDGF-A .MRTWACILL LGGYLANAL AEEAEIPREL IERLARSQIH SINDLQRLIE
 PDGF-B MNRCAW.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDRSIR SFDDLQRLIE
 P1GF-1MPVM RLFPFC..FLQ LLAGLAL...
 P1GF-2MPVM RLFPFC..FLQ LLAGLAL...
 VEGF121M NFLLS..WVH WSLALLLYLH
 VEGF165M NFLLS..WVH WSLALLLYLH
 VEGF189M NFLLS..WVH WSLALLLYLH
 VEGF206M NFLLS..WVH WSLALLLYLH
 Flt4-LM TVLYPEYWKI YKCQLRKGGW

51 100

PDGF-A IDSVGAEDAL ETSLRAGSH AINHVPKRP VPIRRKRSI.....EEAIP
 PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI
 P1GF-1 PAVPPQOW... .ALSAG NGSSEVEVVP FQE.VWGR...
 P1GF-2 PAVPPQOW... .ALSAG NGSSEVEVVP FQE.VWGR...
 VEGF121 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF165 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF189 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 VEGF206 HAKWSQAA... .PMAEG GGQNHHEVVK FMD.VYQR...
 Flt4-L QHNREQANLN SRTEETIKFA AAHYNTEILK SIDNEWRK...

101 150

PDGF-A AVCKTTRTVY EIPRSQVDPT SANFLIWPPC VEVKRCTGCC NTSSVKCQPS
 PDGF-B AECKTTRTEVF EISRLIDRT NANFLVWPPC VEVCRCGGCC NNRRNVQCRPT
 P1GF-1 SYCRALERLV DVVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
 P1GF-2 SYCRALERLV DVVSEYPS.. EVEHMFSPSC VSLLRCTGCC GDENLHCVPV
 VEGF121 SYCHPIETLV DIFQEYPD.. EIEYIFKPC VPLMRCGGCC NDEGLECVPT
 VEGF165 SYCHPIETLV DIFQEYPD.. EIEYIFKPC VPLMRCGGCC NDEGLECVPT
 VEGF189 SYCHPIETLV DIFQEYPD.. EIEYIFKPC VPLMRCGGCC NDEGLECVPT
 VEGF206 SYCHPIETLV DIFQEYPD.. EIEYIFKPC VPLMRCGGCC NDEGLECVPT
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151 200

PDGF-A RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEACAT... ..SN
 PDGF-B QVQLRPVQVR KIEIVRKPKI FKKATVTLED HLAACKETVA AARPVTRSPG
 P1GF-1 ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPER...
 P1GF-2 ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRCECRPLR EKMKPERRR.
 VEGF121 EESNITMQIM RIKPH..QCG .HIGEMSFLQ HNKCECRPKK DRARQEKCD.
 VEGF165 EESNITMQIM RIKPH..QCG .HIGEMSFLQ HNKCECRPKK DRARQEN...
 VEGF189 EESNITMQIM RIKPH..QCG .HIGEMSFLQ HNKCECRPKK DRARQEKKS.
 VEGF206 EESNITMQIM RIKPH..QCG .HIGEMSFLQ HNKCECRPKK DRARQEKKS.
 Flt4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSCRCMSKL DVYRQVHSII

201 250

PDGF-A LNPDRHEEET DVR.....
 PDGF-B GSQEQRATP QTRVTIRTVR VRRPPKGRHR KFKHTHDKTA LKETLGA...
 P1GF-1CGDAVPR R.....
 P1GF-2PKGRCK RRREKQRPD CHLCGDAVPR R.....
 VEGF121KPRR.....
 VEGF165
 VEGF189VRGKCK GQKRKRKRSR YKWSV.....
 VEGF206VRGKCK GQKRKRKRSR YKWSVYVGA RCC.....L MPWSLPGPH
 Flt4-L RRSLPATLPQ CQAANKTCPT NYMNNHICR CLAQEDHFS SDAGDDSTOG

FIG. 10A

| | | | | | |
|---------|-------------|-------------|-------------|------------|------------|
| | 251 | | | | 300 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | CGPCSEERRKH | LFVQDPQTCK | CSCKNITDSRC | KARQLELNER | TCRCDKPRR. |
| VEGF189 | CGPCSEERRKH | LFVQDPQTCK | CSCKNITDSRC | KARQLELNER | TCRCDKPRR. |
| VEGF206 | CGPCSEERRKH | LFVQDPQTCK | CSCKNITDSRC | KARQLELNER | TCRCDKPRR. |
| Flt4-L | FHDICGPNKE | LDEETCQCVC | RAGLRPASC | PHKELDRNSC | QCVCKNKLFP |
| | 301 | | | | 350 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | | | | | |
| VEGF189 | | | | | |
| VEGF206 | | | | | |
| Flt4-L | SQCGANREFD | ENTCQCVCCKR | TCPRNQPLNP | GKCACECTES | PQKCLLKGGK |
| | 351 | | | | 394 |
| PDGF-A | | | | | |
| PDGF-B | | | | | |
| PlGF-1 | | | | | |
| PlGF-2 | | | | | |
| VEGF121 | | | | | |
| VEGF165 | | | | | |
| VEGF189 | | | | | |
| VEGF206 | | | | | |
| Flt4-L | FHHQTCSCYR | RPCTNRQKAC | EPGFSYSEEV | CRCVPSYWKR | PQMS |

FIG. 10

| | | | |
|---------|------------|------------------|-----------------------------------|
| | 1 | | 50 |
| PDGF-A | .MRTWACLLL | LGGCYLAHAL | AEAEIIPREL IERLARSQIH SIRDQLRLLE |
| PDGF-B | MNRCA.LFL | SLCCYLRLVS | AEGDPIPEEL YEMLSHISIR SFDDLQRLH |
| PlGF-1 | | |MPVM RLFPCL..FLQ LLAGLAL... |
| PlGF-2 | | |MPVM RLFPCL..FLQ LLAGLAL... |
| VEGF121 | | |M NFLLS..WVH WSLALLLYLH |
| VEGF165 | | |M NFLLS..WVH WSLALLLYLH |
| VEGF189 | | |M NFLLS..WVH WSLALLLYLH |
| VEGF206 | | |M NFLLS..WVH WSLALLLYLH |
| Flt4-L | | |M TVLYPEYWKM YKQCLRKGGW |
| | 51 | | 100 |
| PDGF-A | IDSVGAEDAL | ETSLRAHGS | AINHVPEKRP VPIRRKRSI.EEAIP |
| PDGF-B | GDP.GEEDGA | ELDLNMTRSH | SGGELES... .LARGRRSLG SLTIAEPAMI |
| PlGF-1 | PAVPPQW.. |ALSAG | NGSSEVEVVP FQE.VWGR.. |
| PlGF-2 | PAVPPQW.. |ALSAG | NGSSEVEVVP FQE.VWGR.. |
| VEGF121 | HAKWSQAA.. |PMAEG | GGQNHHEVVK FMD.VYQR.. |
| VEGF165 | HAKWSQAA.. |PMAEG | GGQNHHEVVK FMD.VYQR.. |
| VEGF189 | HAKWSQAA.. |PMAEG | GGQNHHEVVK FMD.VYQR.. |
| VEGF206 | HAKWSQAA.. |PMAEG | GGQNHHEVVK FMD.VYQR.. |
| Flt4-L | QHNREQANLN | SRTEETIKFA | AAHYNTEILK SIDNEWRK.. |
| | 101 | | 150 |
| PDGF-A | AVCKTRTVIY | EIPRSQVDPT | SANFLIWPPC VEVRKCTGCC NTSSVKCQPS |
| PDGF-B | AECKTRTEVF | EISRRILDR | NANFLVWPPC VEVRKCTGCC NNRNVQCRPT |
| PlGF-1 | SYCRALERLV | DVVSEYPS.. | EVEHMFSPSC VSLLRCTGCC GDNELHCVPV |
| PlGF-2 | SYCRALERLV | DVVSEYPS.. | EVEHMFSPSC VSLLRCTGCC GDNELHCVPV |
| VEGF121 | SYCHPIETLV | DIFQEYPD.. | EIEYIFKPS VPLMRCGGCC NDEGLECVPT |
| VEGF165 | SYCHPIETLV | DIFQEYPD.. | EIEYIFKPS VPLMRCGGCC NDEGLECVPT |
| VEGF189 | SYCHPIETLV | DIFQEYPD.. | EIEYIFKPS VPLMRCGGCC NDEGLECVPT |
| VEGF206 | SYCHPIETLV | DIFQEYPD.. | EIEYIFKPS VPLMRCGGCC NDEGLECVPT |
| Flt4-L | TQCMPREVCI | DVGKEFGV.. | ATNTFFKPPC VSVYRCGGCC NSEGLQCMNT |
| | 151 | | 200 |
| PDGF-A | RVHHRSVKVA | KVEYVRKKPK | LKEVQVRLEE HLEACAT..SN |
| PDGF-B | QVQLRPVQVR | KIEIVRKPI | FKKATVTLED HLACKCETVA AARPVTRSPG |
| PlGF-1 | ETANVTMQLL | KIRSG..DRP | .SYVELTFSQ HVRCECRPLR EKMKPER... |
| PlGF-2 | ETANVTMQLL | KIRSG..DRP | .SYVELTFSQ HVRCECRPLR EKMKPERRR.. |
| VEGF121 | EESNITMQIM | RIKPH..QQQ | .HIGEMSFLQ HNKCECRPKK DRARQEKCD.. |
| VEGF165 | EESNITMQIM | RIKPH..QQQ | .HIGEMSFLQ HNKCECRPKK DRARQEN... |
| VEGF189 | EESNITMQIM | RIKPH..QQQ | .HIGEMSFLQ HNKCECRPKK DRARQEKKS.. |
| VEGF206 | EESNITMQIM | RIKPH..QQQ | .HIGEMSFLQ HNKCECRPKK DRARQEKKS.. |
| Flt4-L | STSYLSKTLF | EITVPLSQGP | .KPVITISFAN HTSCRCMSKL DVYRQVHSII |
| | 201 | | 250 |
| PDGF-A | LNPDRHEET | DVR..... | |
| PDGF-B | GSQEQRATP | QTRVTIRTVR | VRRPPKGGHR KFKHTHDKTA LKETLGA... |
| PlGF-1 | | |CGDAVPR R..... |
| PlGF-2 | | PKGRGK RRREKQRPD | CHLCGDAVPR R..... |
| VEGF121 | | KPRR.. | |
| VEGF165 | | |P |
| VEGF189 | | VRGKGK GQKRKRKSR | YKSWSV.....P |
| VEGF206 | | VRGKGK GQKRKRKSR | YKSWSVYVGA RCC.....L MPWSLPGPH |
| Flt4-L | RRSLPATLPQ | CQAANKTCPT | NYMWNHICR CLAQEDFMFS SDAGDDSTDG |

FIG. 10

MetThrValLeuTyrProGluTyr
 CACCAGTTACGGTCTCTGTCCTCACTGATCAACTCATGACTGTACTCTACCCAGAAATAT
 10 30 50
 TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla
 TGGAAAATGTACAAGTGTCAAGTAAGGAAGGAGGCTGGCAACATAACACAGAACAGGCC
 70 90 110
 AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu
 AACCTCAACTCAAGGACAGAGAGACTATAAAATTTGCTCAGCACATTATAATACAGAG
 130 150 170
 IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys
 ATCTTGAAAAGTATTGATAATGAGTGGACAAAGACTCAATGCATGCCACGGGAGGTGTG
 190 210 230
 IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal
 ATAGATCTGCCAAGGAGTTTGGAGTCCGACAAACACCTTCTTAAACCTCCATGTGTG
 250 270 290
 SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer
 TCCGTCTACAGATGTCCGGTTCCTGCAATAGTACGGGGCTGCACTGCATCAACACCAGC
 310 330 350
 ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys
 ACGAGCTACTCAGCAAGACGTTATTGAAATTACAGTCCCTCTCTCAAGCCCCAAA
 370 390 410
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal
 CCAGTAACAATCAGTTTGGCAATCACACTTCCCTGCCGATCCATCTCTAACTGGATGTT
 430 450 470
 TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln
 TACAGACAAGTTCATTCCATATTAGAGCTTCCCTGCCAGCAACACTACCACAGTGTGAC
 490 510 530
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu
 GCAGCGAACAAGACCTGCCCCACCAATTACATGTGGAATATCACATCTGCAGATGCCCTG
 550 570 590
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis
 CCTCAGCAACATTTTATGTTTCTCTCGATGCTGGAGATCACTCAACAGATGGATTCCAT
 610 630 650
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla
 CACATCTGTGGACCAACAAGGAGCTGGATGAAGAGACCTGTCACTTCTCTCCAGAGCG
 670 690 710
 GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys
 GGGCTTCGGCCCTGCCAGCTGTGGACCCACAAAGAACTAGACAGAACTCATGCCAGTGT
 730 750 770
 ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn
 GTCTGTAAAAACAACCTCTTCCCGCAATGTGGGGCCACCGAGAATTGATCAAAAAC
 790 810 830
 ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys
 ACATGCCAGTGTGTATGTAAAAGAACCTGCCCCAGAAATCAACCCCTAAATCTCGAAAA
 850 870 890
 CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis
 TGTGCTGTGAATGTACAGAAAGTCCACAGAAATGCTTGTAAAAGGAAAGAGTTCCAC
 910 930 950
 HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro
 CACCAACATGCAGCTGTACAGACGGCCATGTACCAACCCGACAGCCCTGTGAGCCA
 970 990 1010
 GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln
 GGATTTTCATATACCAAGAACTCTGCTGCTGCTCCCTTCATATTGGAAGACCACAA
 1030 1050 1070
 MetSerEnd
 ATGAGCTAAGATTGTACTGTTTCCAGTTCATCGATTTTCTATTATCGAAAACCTGTGTTG
 1090 1110 1130

FIG. 9B

FIG. 9A

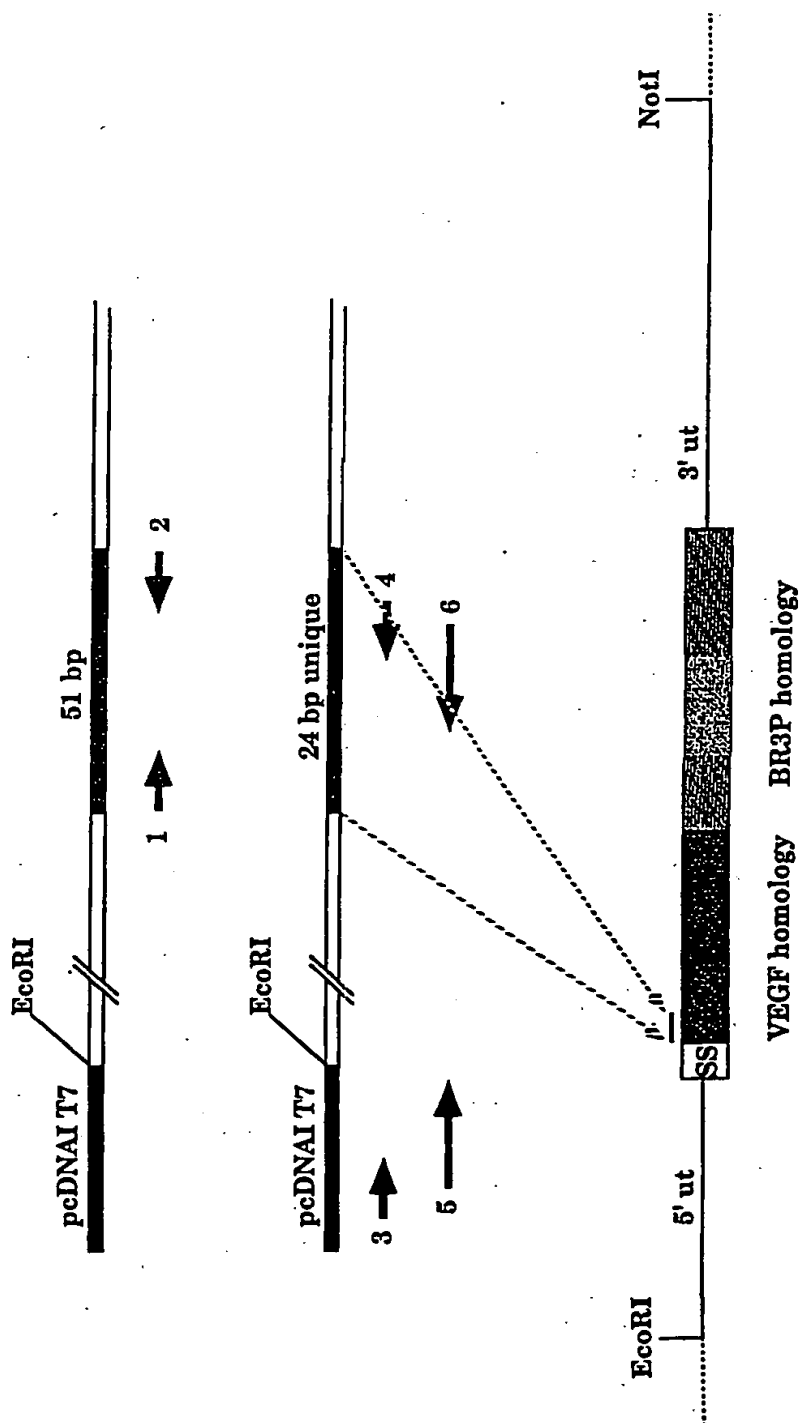
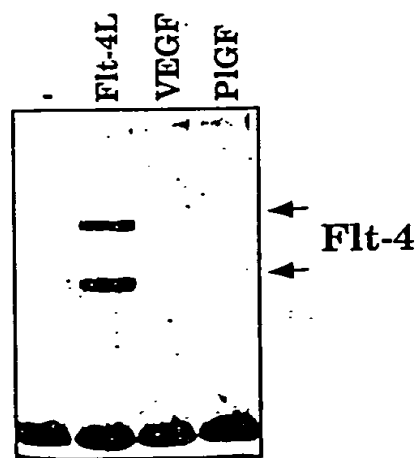


FIGURE 8



08 585895

FIGURE 7

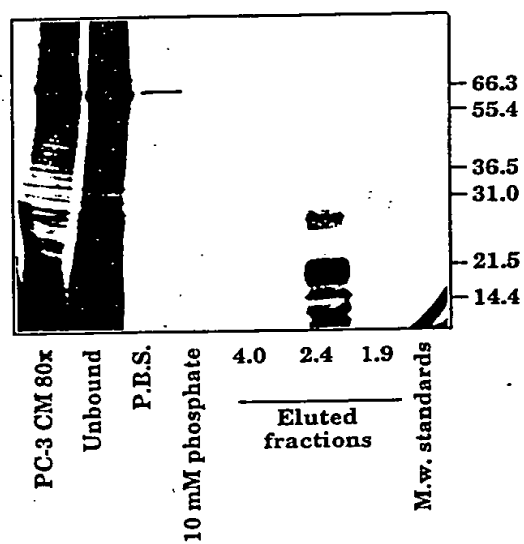


FIGURE 7

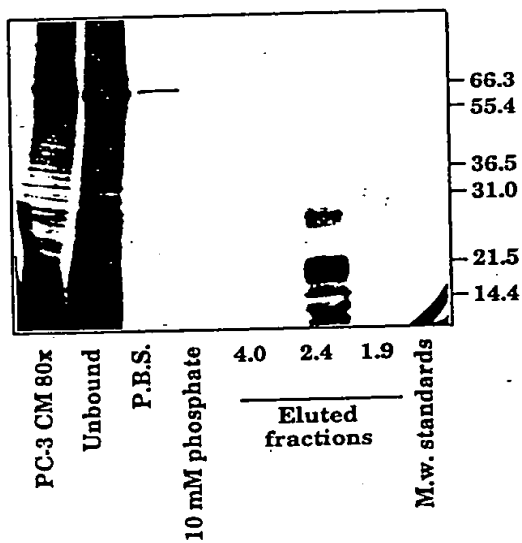
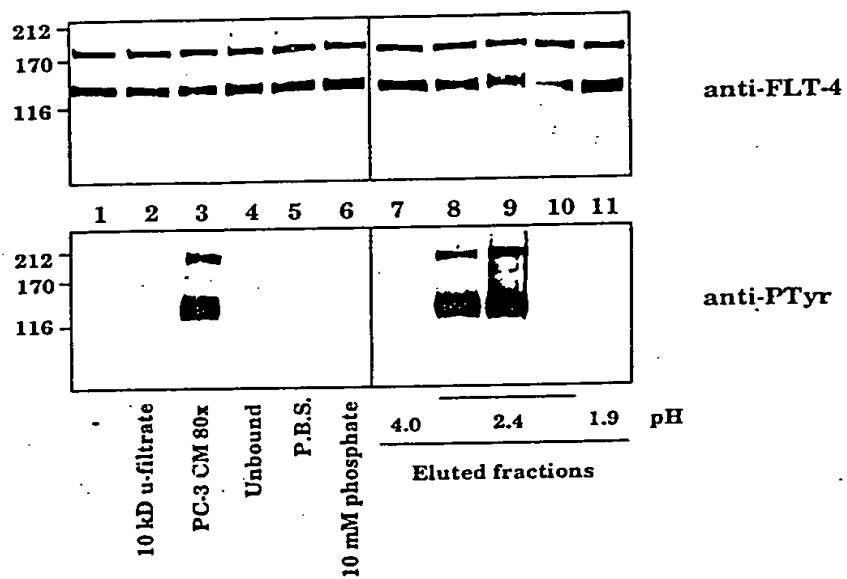


FIGURE 6



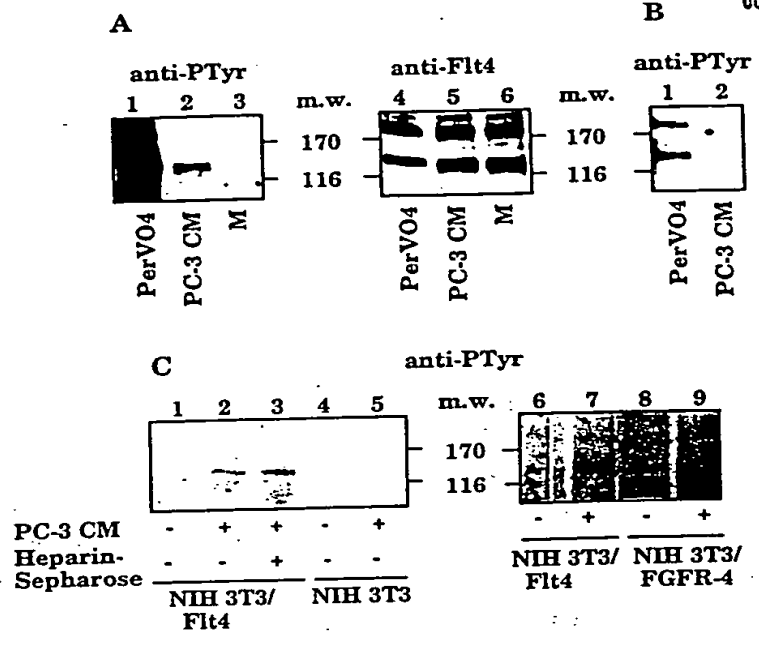


FIGURE 5

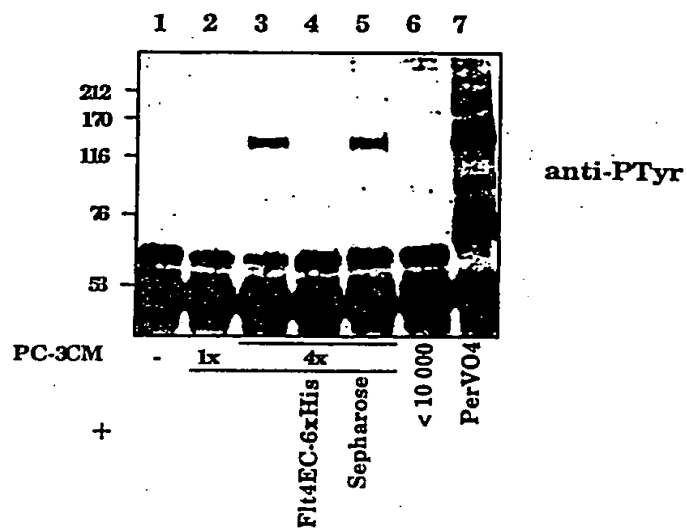


FIGURE 4

Figure 3

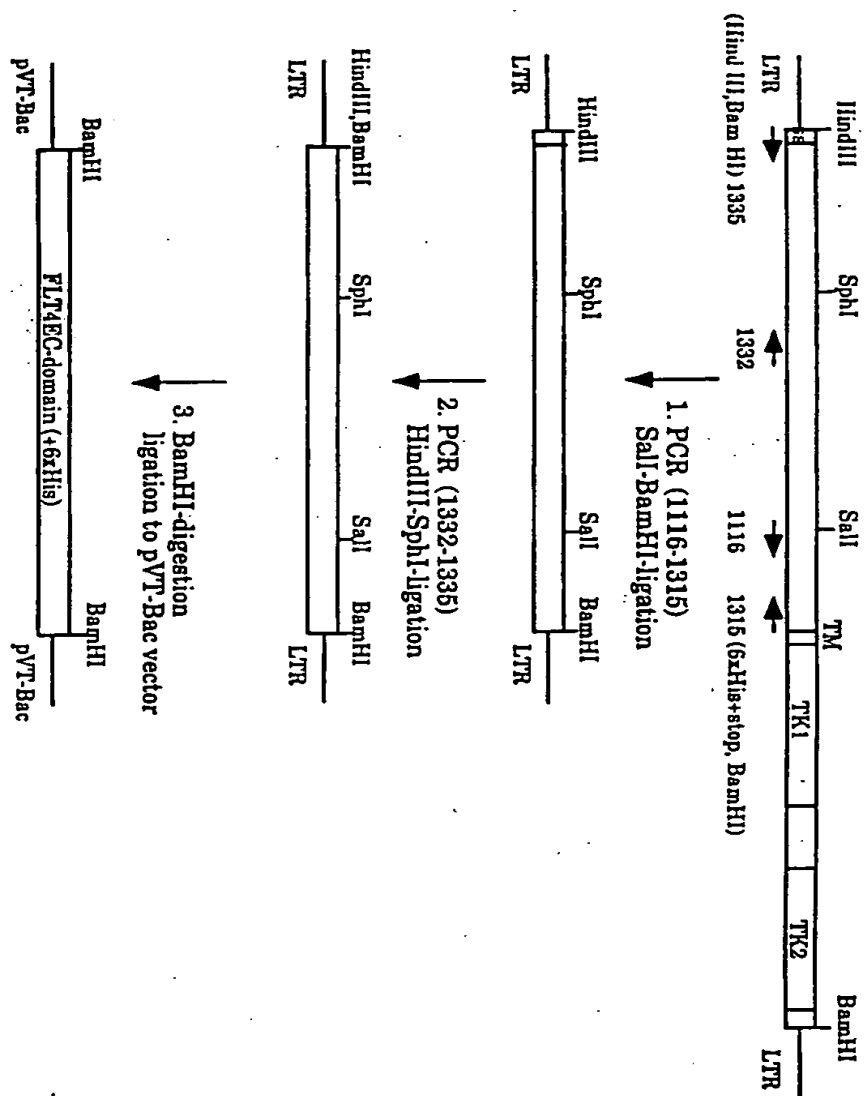




FIGURE 2

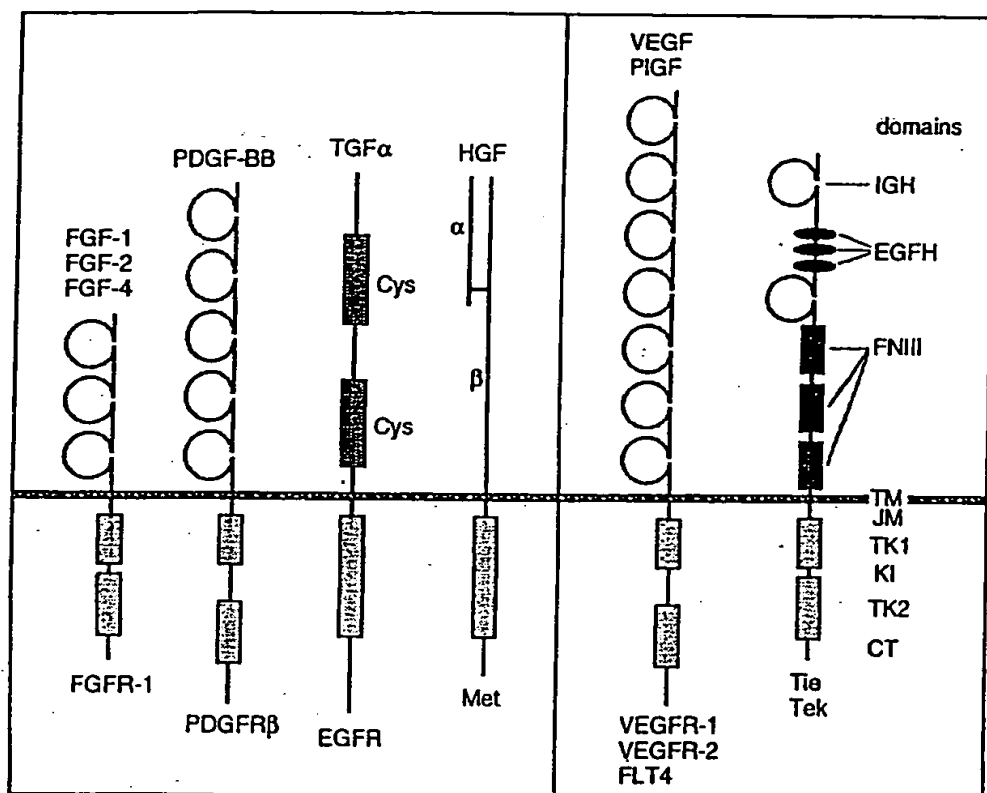


FIGURE 1

-51-

12. The fragment according to claim 8 comprising approximately amino acids 1-180 shown in SEQ ID NO: 33.

Seq 33

13. A purified and isolated nucleic acid encoding the fragment of claim 12.

14. An antibody which is specifically reactive with the Flt4 ligand.

15. An antibody of claim 14 which is a monoclonal antibody.

16. A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

add B6

add C12

add D12

CLAIMS

1. A purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.
2. A purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.
3. A purified and isolated nucleic acid encoding the peptide according to claim 2.
4. The nucleic acid according to claim 3 having the sequence shown in SEQ ID NO: 32.
5. A vector comprising the nucleic acid according to claim 4.
6. The vector according to claim 5, wherein said vector is plasmid pFLT4-L, deposited as ATCC accession No. 97231.
7. A host cell comprising the vector according to claim 6.
8. A fragment of the purified and isolated polypeptide according to claim 2, said fragment being capable of specifically binding to an Flt4 receptor tyrosine kinase.
9. The fragment according to claim 8 having an apparent molecular weight of 23 kD under reducing conditions.
10. The fragment according to claim 8 comprising approximately amino acids 1-120 of SEQ ID NO: 33.
11. A purified and isolated nucleic acid encoding the fragment of claim 10.

Sub B1

Sub B3

Sub B4
C5

Sub C6

Sub B5

Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
115 120 125
Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
130 135 140
Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
145 150 155 160
Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
165 170 175
Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
180 185 190
Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
195 200 205
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
210 215 220
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
225 230 235 240
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
245 250 255
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
260 265 270
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
275 280 285
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
290 295 300
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCCATCT

22

| | |
|---|------|
| CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC | 774 |
| Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys | |
| 200 205 210 | |
| CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC | 822 |
| Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn | |
| 215 220 225 230 | |
| CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC | 870 |
| Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys | |
| 235 240 245 | |
| CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA | 918 |
| Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr | |
| 250 255 260 | |
| GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA | 966 |
| Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln | |
| 265 270 275 | |
| ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT | 1014 |
| Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys | |
| 280 285 290 | |
| GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA | 1062 |
| Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser | |
| 295 300 305 310 | |
| TAT TGG AAA AGA CCA CAA ATG AGC TAA GATTGTACTG TTTCCAGTT | 1109 |
| Tyr Trp Lys Arg Pro Gln Met Ser | |
| 315 | |
| CATCGATTIT CTATTATGGA AAACGTGTT G | 1140 |

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

| | |
|---|-----------------|
| Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu | -32 -30 -25 -20 |
| Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser | -15 -10 -5 |
| Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu | 1 5 10 15 |
| Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro | 20 25 30 |
| Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn | 35 40 45 |
| Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys | 50 55 60 |
| Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu | 65 70 75 80 |
| Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys | 85 90 95 |
| Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser | 100 105 110 |

(ii) MOLECODE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 37..1089

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 133..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

| | | |
|---|-------------------------|-----|
| GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC | ATG ACT GTA CTC TAC CCA | 54 |
| | Met Thr Val Leu Tyr Pro | |
| | -32 -30 | |
| GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA | | 102 |
| Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln | | |
| -25 -20 | -15 | |
| CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA | | 150 |
| His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile | | |
| -10 -5 1 5 | | |
| AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT | | 198 |
| Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp | | |
| 10 15 20 | | |
| AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT | | 246 |
| Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp | | |
| 25 30 35 | | |
| GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA | | 294 |
| Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro | | |
| 40 45 50 | | |
| TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG | | 342 |
| Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu | | |
| 55 60 65 70 | | |
| CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA | | 390 |
| Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu | | |
| 75 80 85 | | |
| ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT | | 438 |
| Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe | | |
| 90 95 100 | | |
| GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA | | 486 |
| Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg | | |
| 105 110 115 | | |
| CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG | | 534 |
| Gln Val His Ser Ile Ile Arg Ser Leu Pro Ala Thr Leu Pro Gln | | |
| 120 125 130 | | |
| TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT | | 582 |
| Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn | | |
| 135 140 145 150 | | |
| CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT | | 630 |
| His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp | | |
| 155 160 165 | | |
| GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC | | 678 |
| Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn | | |
| 170 175 180 | | |
| AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT | | 726 |
| Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu | | |
| 185 190 195 | | |

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATT AGGTGACAC 19

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTCGCT GCAGC 25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTTGTAGTGT GCTGCAGCGA ATTT 24

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAAG C 21

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1140 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 8 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 219 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT
GTGGTGGAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG
TGTGAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC

60

120

180

219

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid

Thr Glu Ile Leu Lys
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 7 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1 5 10 15
Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
 20 25 30
His Arg Gln Glu Ser Gly Phe Arg
 35 40

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 60 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGCCTGTG ATGTGCACCA

20

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
ACATGCATGC CCCGCCGGTC ATCC 24
- (2) INFORMATION FOR SEQ ID NO:4:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
CGGAATTCCTCC CATGACCCCA AC 22
- (2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33
- (2) INFORMATION FOR SEQ ID NO:6:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
ATTTAGGTGA CACTATA 17
- (2) INFORMATION FOR SEQ ID NO:7:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34
- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids



- 40 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari
Joukov, Vladimir
- (ii) TITLE OF INVENTION: Receptor Ligand
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gass, David A.
(B) REGISTRATION NUMBER: 38,153
(C) REFERENCE/DOCKET NUMBER: 28113/33072
- (ix) TELECOMMUNICATION INFORMATION:
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(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTGCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

isolated (as in the other examples), and 8 micrograms of the RNA was electrophoresed and blot-hybridized with a mixture of the VEGF, VEGF-B and VEGF-C probes (see Fig. 12). The results show that hypoxia strongly induces VEGF-A mRNA expression (compare lanes - and +), both in low and high glucose, but has no significant effect on the VEGF-B mRNA levels. The VEGF-C mRNA isolated from hypoxic cells runs slightly faster in gel electrophoresis and an extra band of faster mobility can be seen below the upper mRNA band. This observation suggests that hypoxia affects VEGF-C RNA processing. One explanation for this observation is that VEGF-C mRNA splicing is altered, affecting the VEGF-C open reading frame and resulting in an alternative VEGF-C protein being produced by hypoxic cells. Such alternative forms of VEGF-C and VEGF-C-encoding polynucleotides are contemplated as an aspect of the invention.

Deposit of Biological Materials: Plasmid FLT4-L has been deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville MD ²⁰⁸⁵² 20952 (USA), pursuant to the provisions of the Budapest Treaty, and has been assigned a deposit date of 24 July 1995 and ATCC accession number 97231.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art. Accordingly, only such limitations as appear in the appended claims should be placed on the invention.

In order to determine the chromosomal localization of the human VEGF-C gene, DNAs from human rodent somatic cell hybrids containing defined sets of human chromosomes were analysed by Southern blotting and hybridization with the VEGF-C cDNA probe. Among 24 DNA samples on the hybrid panel, representing different human chromosomes, human-specific signals were observed only in hybrids which contained human chromosome 4. The results were confirmed by PCR of somatic cell hybrid DNA using VEGF-C specific primers, where amplified bands were obtained only from DNAs containing human chromosome 4.

A genomic P1 plasmid for VEGF-C was isolated using specific primers and PCR and verified by Southern blotting and hybridization using a VEGF-C specific cDNA probe. The chromosomal localization of VEGF-C was further studied using metaphase FISH. Using the P1 probe for VEGF-C in FISH a specific hybridization to the 4q34 chromosomal band was detected in 40 out of 44 metaphases (Fig. 17). Double-fluorochrome hybridization using a cosmid probe specific for the aspartylglucosaminidase (AGA) gene showed that VEGF-C is located just proximal to the AGA gene previously mapped to the 4q34-35 chromosomal band.

Biotin labelled VEGF-C P1 and digoxigenin labeled AGA cosmid probes were hybridized simultaneously to metaphase chromosomes. This experiment demonstrated that the AGA gene is more telomerically located than the VEGF-C gene. The foregoing example demonstrates the utility of polynucleotides of the invention as chromosomal markers.

EXAMPLE 18

Effect of glucose concentration and hypoxia on VEGF, VEGF-B and VEGF-C mRNA levels in C6 glioblastoma cells

Confluent cultures of C6 cells (ATCC CCL 107) were grown on 10 cm diameter tissue culture plates containing 2.5 ml of DMEM and 5 % fetal calf serum plus antibiotics. The cultures were exposed for 16 hours to normoxia in a normal cell culture incubator containing 5 % CO₂ (Fig. 18: lanes marked -) or hypoxia (Fig. 18: lanes marked +) by closing the culture plates in an airtight glass chamber and burning a piece of wood inside until the flame was extinguished due to lack of oxygen. Polyadenylated RNA was

domain was used as a probe in Southern blotting and hybridization analysis of the somatic cell hybrid DNAs as instructed by the supplier (Bios Laboratories).

The cell lines for fluorescence *in situ* hybridization (FISH) were obtained from the American Type Culture Collection (Rockville, MD).

5 Purified DNA from P1 clones 7660 and 7661 (VEGF-C) (Genome Systems, Inc., St. Louis, MO) were confirmed positive by Southern blotting of Eco RI-digested DNA followed by hybridization with the VEGF-C cDNA. The P1 clones were then labelled by nick translation either with biotin-11-dUTP, biotin-14-ATP (Sigma Chemical Co., St. Louis, MO) or digoxigenin 11-dUTP
10 (Boehringer Mannheim GmbH, Mannheim, Germany) according to standard protocols. PHA-stimulated peripheral blood lymphocyte cultures were treated with 5-bromodeoxyuridine (BrdU) at an early replicating phase to induce G-banding. See Takahashi *et al.*, *Human Genet.*, 86:14-16 (1995); Lemieux *et al.*, *Cytogenet. Cell Genet.*, 59:311-12 (1992). The FISH procedure was
15 carried out in 50% formamide, 10% dextran sulphate in 2x SSC using well-known procedures. See, e.g., Rytönen *et al.*, *Cytogenet. Cell Genet.*, 68:61-63 (1995); Lichter *et al.*, *Proc. Natl. Acad. Sci. USA*, 85:9664-68 (1988). Repetitive sequences were suppressed with 50-fold excess of Cot-1
C DNA (BRL, ^{Haitersburg} Gaithersburg, MD) compared with the labeled probe. Specific
20 hybridization signals were detected by incubating the hybridized slides in labelled antidigoxigenin antibodies, followed by counterstaining with 0.1mmol/L 4,6-diamino-2-phenylindole. Probe detection for two-color experiments was accomplished by incubating the slides in fluorescein isothiocyanate (FITC)-anti-digoxigenin antibodies (Sigma Chemical Co.) and
25 Texas red-avidin (Vector Laboratories, Burlingame, CA) or rhodamine-anti-digoxigenin and FITC-avidin.

Multi-color digital image analysis was used for acquisition, display and quantification of hybridization signals of metaphase chromosomes. The system contains a PXL camera (Photometrics Inc., Tucson, AZ) attached
30 to a PowerMac 7100/Av workstation. IPLab software controls the camera operation, image acquisition and Ludl Filter wheel. At least 50 nuclei were scored. Overlapping nuclei and clusters of cells were ignored. A slide containing normal lymphocyte metaphase spreads and interphase nuclei was included in each experiment to control for the efficiency and specificity of the
35 hybridization.

example of typical phase contrast and fluorescent microscopic fields of cultures stimulated with medium from mock-transfected or VEGF-C transfected cells is shown in Fig. 15B. Daily addition of 1 ng of FGF2 into the wells resulted in the migration of approximately twice the number of cells when compared to the stimulation by CM from VEGF-transfected cells.

EXAMPLE 16

VEGF-C Is Expressed In Multiple Tissues

Northern blots containing 2 micrograms of isolated poly(A)⁺ RNA from multiple human tissues (blot from Clontech) were probed with radioactively labelled insert of the 2.0 kb VEGF-C cDNA clone. Northern blotting and hybridization analysis showed that the 2.4 kb RNA and smaller amounts of a 2.0 kb mRNA are expressed in multiple human tissues, most prominently in the heart, placenta, muscle, ovary and small intestine (Fig. 16A). Very little VEGF-C RNA was seen in the brain, liver or thymus and peripheral blood leukocytes (pbl) appeared negative. A similar analysis of RNA from human fetal tissues (Fig. 16B) shows that VEGF-C is highly expressed in the kidney and lung and to a lesser degree in the liver, while essentially no expression is detected in the brain. Interestingly, VEGF expression correlates with VEGF-C expression in these tissues, whereas VEGF-B is ^{highly} expressed in all tissues ^{analysed}.

EXAMPLE 17

The VEGF-C Gene Localizes To Chromosome 4q34

A DNA panel of 24 interspecies somatic cell hybrids, which had retained one or two human chromosomes, was used for the chromosomal localization of the VEGF-C gene (Bios Laboratories, Inc., New Haven, CT). Primers were designed to amplify an about 250 bp fragment of the VEGF-C gene from somatic cell hybrid DNA. The primers and conditions for polymerase chain reaction (PCR) were 5'-TGAGTGATTGTAGCTGCTGTG-3' (forward) [SEQ ID NO:34] and 5'-TATTGCAGCAACCCCCACATCT-3' (reverse) [SEQ ID NO:35] for VEGF-C (94°C, 60s/62°C, 45s/72°C, 60s). The PCR products were evaluated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining in ultraviolet light. [α -³²P]-dCTP-labelled cDNA inserts of a plasmid representing the complete VEGF-C coding

For the migration assays, the cells were allowed to attach inside a plastic ring (1 cm diameter) placed on top of the first collagen layer. After 30 min., the ring was removed and unattached cells were rinsed away. A second layer of collagen and a layer of growth medium (5% newborn calf serum (NCS)), solidified by 0.75% low melting point agar (FMC BioProducts, Rockland, ME), were added. A well (3 mm diameter) was punched through all the layers on both sides of the cell spot at a distance of 4 mm, and the sample or control media were pipetted daily into the wells. Photomicrographs of the cells migrating out from the spot edge were taken after six days through an Olympus CK 2 inverted microscope equipped with phase-contrast optics. The migrating cells were counted after nuclear staining with the fluorescent dye bisbenzimidazole (1 mg/ml, Hoechst 33258, Sigma).

Fig. 15A depicts a comparison of the number of cells migrating at different distances from the original area of attachment towards wells containing media conditioned by the non-transfected (control) or transfected (mock; VEGF-C; VEGF) cells, 6 days after addition of the media. The number of cells migrating out from the original ring of attachment was counted in five adjacent 0.5 mm x 0.5 mm squares using a microscope ocular lens grid and 10x magnification. Cells migrating further than 0.5 mm were counted in a similar way by moving the grid in 0.5 mm steps. The experiments were carried out twice with similar results, and medium values from the one of the experiments are presented with standard error bars. The photographs in Fig. 15B depict phase-contrast microscopy and fluorescent microscopy of the nuclear staining of BCE cells migrating towards the wells containing media conditioned by the mock-transfected cells or by VEGF-C - transfected cells. The areas shown is approximately 1mm x 1.5mm, and arrows indicate the borders of the original ring of attachment.

After 6 days of treatment, the cultures were stained and cells at different distances outside of the original ring of attachment were counted using fluorescent nuclear staining and detection with a fluorescence microscope equipped with a grid. A comparison of the numbers of migrating cells in successive 0.5 mm x 0.5 mm areas is shown in Fig 15A. As can be seen from the columns, VEGF-C-containing CM stimulated cell migration more than medium conditioned by the non-transfected or mock-transfected cells but less than medium from cells transfected with a VEGF expression vector. An

nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH₂-terminal sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in carboxy-terminal truncations of its protein product. The activities of such truncated forms are ^{assayed} ~~assayed~~ by, for example, studying Flt4 autophosphorylation induced by the truncated proteins when applied to cultures of cells, such as NIH3T3^{3T3} cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin *et al.*, *Growth Factors* 8:245-252 (1993)) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues of the secreted VEGF-C protein lacking the signal sequence, and apparently within the first approximately 120 amino acid residues.

On the other hand, the difference between the molecular weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNA sequences are determined from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art, such as heteroduplex analysis of cDNA and genomic DNA, which are subsequently be characterized.

Parklawn Drive, Rockville, MD 20852 as accession number 97231.

However, the predicted molecular weight of the mature protein product deduced from this reading-frame is 35881 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4-L mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues underlined in Fig. 10).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, Gene 88, 133-140, 1990), as depicted in Fig. 9A. Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

Thus, the Flt4-L mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells. One uses antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or

PDGF/VEGF family of growth factors, as shown in Figure 10.

EXAMPLE 11

Stimulation of Flt4 autophosphorylation by the protein product of the Flt4 ligand vector

5 The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector
containing the open reading frame encoding the sequence shown in Fig: 9B⁻
(SEQ ID NO: 32) was cut out from the vector using *HindIII* and *NotI*
restriction enzymes, isolated from a preparative agarose gel and ligated to the
corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7
10 vector containing the above cloned insert was transfected into 293-EBNA cells
(Invitrogen) using the calcium phosphate transfection method (Sambrook et al.,
Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory
Press, 1989). About 48 hours after transfection the medium of the transfected
cells was changed to DMEM medium lacking fetal calf serum and incubated
15 for 36 h. The thus conditioned medium was then collected, centrifuged at
5000 x g for 20 minutes, the supernatant was concentrated 5-fold using
Centriprep 10 (Amicon) and used to stimulate NIH3T3^{3T3} cells expressing
LTRFlt4l, as in Example 4. The cells were lysed, immunoprecipitated using
C anti-Flt4 antiserum and analysed by Western blotting using anti-
20 phosphotyrosine antibodies.

As can be seen from Fig. 11, lanes 1 and 3, the conditioned
medium from two different dishes of the transfected cells stimulated Flt4
autophosphorylation in comparison with the medium from mock-transfected
cells, which gave only background levels of phosphorylation of the Flt4
25 receptor (lane 2). When the concentrated conditioned medium was pre-
C absorbed with 20 μ l of a ^{slurry} of Flt4EC domain coupled to Sepharose (see
example 4), no phosphorylation was obtained (lane 4), showing that the
activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand.
Thus, these results demonstrate that the Flt4-L plasmid vector clone having an
30 approximately 2.1 kb insert and containing the open reading frame shown in
Fig. 9B is expressed into a Flt4 ligand in cells transfected with the Flt4-L
expression vector clone, and thus is biologically active. The sequence encoded
by that open reading frame is shown in SEQ ID NO: 33. Plasmid pFLT4-L
has been deposited with the American Type Culture Collection, 12301

NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 31)
(sense-primer corresponding to nucleotides 2179-2199 of the pcDNAI vector).
The amplified product was subjected to digestion with *EcoRI* (Boehringer
Mannheim) to remove the portion of the DNA sequence amplified from the
5 pcDNAI vector and the resulting 153 bp fragment encoding the 5' end of the
Flt4 ligand was labeled with [³²P]-dCTP using the Klenow fragment of *E. coli*
DNA polymerase I (Boehringer Mannheim). That fragment was used as a
probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the
10 radioactively labeled probe at 42 °C for 20 hours in a solution containing 50%
formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml
denatured salmon sperm DNA. Filters were washed twice in 1x SSC, 0.1%
SDS for 30 minutes at room temperature, then twice for 30 minutes at 65 °C
and exposed overnight.

15 On the basis of autoradiography, 10 positive recombinant
bacterial colonies hybridizing with the probe were chosen from the library.
C Plasmid DNA was purified from these colonies and analysed by *EcoRI* and
NotI digestion and agarose gel electrophoresis followed by ethidium bromide
staining. The ten plasmid clones were divided into three groups on the basis
20 of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb,
respectively. Inserts of plasmids from each group were sequenced using the
T7 oligonucleotide as a primer and walking primers for subsequent sequencing
reactions.

Sequence analysis showed that all clones contain the open
25 reading frame encoding the NH2-terminal sequence of the Flt4 ligand.
Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the
signal sequence (Fig. 9A, SS). The 5' end of the 1.7 kb clone began within
the signal sequence-encoding portion. Dideoxy sequencing was continued
using walking primers in the downstream direction. An 1140 nucleotide
30 portion of the sequence of the longest clone is shown in Figure 9B. As can be
seen in that figure, after the putative signal sequence the open reading frame
terminates in a TAA stop codon 318 amino acid residues further downstream
C from the ³³32 amino acid signal sequence. When compared with sequences in
the GenBank Database, the predicted protein product of this reading frame was
35 found to be homologous with the predicted amino acid sequences of the

The beginning of the sequence represents the pcDNAI vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand. The cloning of the 5' end of the Flt4 cDNA, as described in the preceding two examples, is depicted schematically in Fig. 9A.

EXAMPLE 9

Amplification of the 3'-end of cDNA encoding the Flt4 ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the FLT4-L clones. The sense-strand primer 5'-ACAGAGAACAGGCCAACC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNAI vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1°C every two cycles from 72°C to 52°C, at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72°C for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNAI vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

EXAMPLE 10

Screening the PC-3 cell cDNA library using the 5' PCR fragment of Flt4 ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID

PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGGTTGTAGTGTGCTG-3' (SEQ ID NO: 19), which is the antisense-strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20), and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21), corresponding to the T7 RNA promoter of the pcDNAI vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, *et al.*, *Nucl. Acids Res.*, 19: 4008 (1991), incorporated by reference herein. The annealing temperature of the two first cycles was 62 °C and subsequently the annealing temperature was decreased in every other cycle by 1 °C until a final temperature of 53 °C was reached, at which temperature 16 additional cycles were conducted. Annealing time was 1 minute and extension at each cycle was conducted at 72 °C for 1 minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand, and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNAI vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72 °C to 66 °C and continuing with 18 additional cycles at 66 °C. The annealing time was 1 minute and extension at each cycle was carried out at 72 °C for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analysed and they contained the sequence 5'-

TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGT
AACGGCCGCCAGTGTGGTGGGAATTCGACGAACTCATGACTGTACTCT
ACCCAGAATATTGGAAAATGTACAAGTGTCAGCTAAGGCAAGGAGGC
TGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAGGACAGAAG
AGACTATAAAATTCGCTGCAGCACACTACAAC- 3' (SEQ ID NO: 25).

lysates were centrifuged for 20 minutes at 15,000 x g. The supernatants were incubated for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus described in Example 2 and also in Pajusola, *et al. Oncogene* 8: 2931-2937, (1993), incorporated by reference herein.

5 After a 2 hour incubation in the presence of anti-Flt4 antiserum, protein A-Sepharose (Pharmacia) was added and incubation was continued for 45 minutes with rotation. The immunoprecipitates were washed three times with the immunoprecipitation buffer and twice with 10 mM Tris, pH7.5 before
10 analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr; PY20) were purchased from Transduction Laboratories (Lexington, Kentucky). In some cases, the filters were restained with a second antibody after stripping. The stripping of
15 the filters was done for 30 minutes at 50°C in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in Figure 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4-
C expressing NIH^{3T3} cells were treated with the indicated preparations of
20 media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the
25 mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of Fig. 4, see also Figure 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in Figure 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15 ul of
C 30 conditioned medium ^{were} separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (Fig. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in Figure 4, lane 1.

As shown in Figure 4, lane 3, stimulating activity was
35 considerably increased when the PC-3 conditioned medium was concentrated

above (the *HindIII* site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the *SphI* site is in Flt4 cDNA). The resultant Flt4EC insert was then ligated as a *BamHI* fragment into the *BamHI* site in the pVTBac plasmid as disclosed in Tessier *et al.*, *Gene* 98: 177-183 (1991),
5 incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection. Recombinant virus was purified, amplified and
10 used for infection of High-Five cells (Invitrogen, San Diego, CA) using methods standard in the art. The Flt4 extracellular domain (Flt4EC) was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-
15 terminus of the recombinant Flt4 extracellular domain.

EXAMPLE 4

Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435
20 from the American Type Culture Collection and cultured as instructed by the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal calf serum. In order to prepare the conditioned media, confluent PC-3 cells were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the absence of fetal bovine serum. Medium was then cleared by centrifugation at 10,000 g
25 for 20 minutes. The medium was then screened to determine its ability to induce tyrosine phosphorylation of Flt4 by exposure to NIH^{3T3} cells which
C had been transfected with Flt4-encoding cDNA using the pLTRFlt4l vector. C For receptor stimulation experiments, subconfluent NIH^{3T3} cells were starved overnight in serum-free DMEM medium (GIBCO) containing 0.2% BSA. The
30 cells were stimulated with the conditioned media for 5 minutes, washed twice with cold PBS containing 100 uM vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole, England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 1 mM vanadate) for receptor immunoprecipitation analysis. The

SphI fragment of the S2.5 plasmid. The resulting vector was digested with *EcoRI* and *ClaI* and ligated to a 138 bp PCR fragment amplified from the 0.6 kb *EcoRI* fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of Flt4s shown in Figure 1 of Pajusola *et al.*,
5 *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-
CGGAATTC³CC CATGACCCCA AC-3' (SEQ ID NO: 4) (forward, *EcoRI*
site underlined) and 5'-CCATCGAT³G³G ATCCTACCTG AAGCCGCTTT
CTT-3' (SEQ ID NO: 5) (reverse, *ClaI* site underlined). The coding domain
was completed by ligation of the 1.2 kb *EcoRI* fragment (base pairs 2535-3789
10 of sequence X68203) into the above construct. The complete cDNA was
subcloned as a *HindIII-ClaI*(blunted) fragment (this *ClaI* site was also included
in the 3' primer used to construct the 3' end of the coding sequence) to the
pLTRpoly expression vector reported in Mäkelä *et al.*, *Gene*, 118: 293-294
(1992) (Genbank accession number X60280), incorporated by reference herein,
15 using its *HindIII-Acc I*(blunted) restriction sites.

The long form of Flt4 was produced by replacing the 3'-end of
the short form as follows: The 3' region of the Flt4l cDNA was PCR-
amplified using a gene specific and a pGEM 3Z vector specific (SP6 promoter)
oligonucleotide 5'-ATTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse
20 and forward primers, respectively, and an Flt4l cDNA clone containing a 495
bp *EcoRI* fragment extending downstream of the *EcoRI* site at nucleotide 3789
of the Genbank X68203 sequence (the sequence downstream of this *EcoRI* site
is deposited as the Flt4 long form 3' sequence having Genbank accession
number S66407). The gene specific oligonucleotide contained a *BamHI*
25 restriction site located right after the end of the coding region. The sequence
of that (reverse primer) oligonucleotide was 5'-
CCATCGAT³GGAT³CCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO: 7)
(*BamHI* site is underlined). The PCR product was digested with *EcoRI* and
BamHI and transferred in frame to LTRFlt4s vector fragment from which the
30 coding sequences downstream of the *EcoRI* site at base pair 2535 (see
sequence X68203) had been removed by *EcoRI-BamHI* digestion. Again, the
coding domain was completed by ligation of the 1.2 kb *EcoRI* fragment (base
pairs 2535-3789 of sequence X68203) back into the resulting construct.

containing base pairs 56-2534 of the Flt4s into the *EcoRI* site of the pSP73 vector (Promega, Madison, WI).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRLW). Poly(A)⁺ RNA was isolated from the HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGTCCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 ^{filters} (Amicon Inc., Beverly, MA). Circularization was made in a total volume of 150 ul. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16°C for 16 hours. Fifteen μ l of this reaction mix was used in a standard 100 ul PCR reaction containing 100 ng of specific primers including *SacI* and *PstI* restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 4 minutes). The PCR mixture was treated sequentially with the *SacI* and *PstI* restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an *SphI* digested PCR fragment amplified using reverse transcription-PCR of poly(A)⁺ RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2)(forward primer, *SphI* site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCCGCCGGT CATCC-3' (SEQ ID NO: 3) (reverse primer, *SphI* site underlined) to the 5' end of the S2.5 fragment, thus replacing unique

and VEGF-C thus increase our understanding of the complexity of the specific and redundant positive signals for endothelial cells involved in vasculogenesis, angiogenesis, permeability and perhaps also other endothelial functions.

C Also described herein is the localization of the VEGF-C gene
5 in human chromosomes by analysis of somatic cell hybrids and fluorescence *in situ* hybridization (FISH). Southern blotting and polymerase chain reaction analysis of somatic cell hybrids and fluorescence *in situ* hybridization of metaphase chromosomes was used to assess the chromosomal localization of the VEGF-C gene. The VEGF-C gene was located on chromosome 4q34,
10 close to the human aspartylglucosaminidase gene previously mapped to 4q34-35. The VEGF-C locus in 4q34 is a candidate target for mutations leading to vascular malformations or cardiovascular diseases. Expression studies by Northern blotting and hybridization show abundant VEGF-C expression in heart and skeletal muscle; other tissues, such as lung and kidney,
C 15 also express these genes. Whereas PlGF is predominantly expressed in the placenta, the expression patterns of the three VEGFs overlap in many tissues, which suggests that they may form heterodimers and interact to exert their physiological functions.

C 20 Targeted mutagenesis leading to inactivation of the VEGF receptor loci in the mouse genome have shown that VEGFR-1 is necessary for the proper organization of endothelial cells forming the vascular endothelium, while VEGFR-2 is necessary for the generation of both endothelial and hematopoietic cells. This suggests that the four genes of the VEGF family can be targets for mutations leading to vascular malformations or cardiovascular
25 diseases.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands and ligand-encoding nucleic acids according to the invention are shown.

EXAMPLE 1

30 Production of pLTRFlt4l expression vector

Construction of the LTR-Flt4l vector is schematically shown in Figure 2. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola *et al.*, *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein,

herein further suggests that this gene product also is involved in the maintenance of the differentiated functions of the lymphatic endothelium where VEGFR-3 is expressed (Kaipainen et al., 1995). Lymphatic capillaries do not have well formed basal laminae and an interesting possibility remains that the silk-like BR3P motif is involved in producing a supramolecular structure which could regulate the availability of VEGF-C in tissues. However, as shown here, VEGF-C also activates VEGFR-2, which is abundant in proliferating endothelial cells of vascular sprouts and branching vessels of embryonic tissues, but decreased in adult tissues. Millauer et al., *Nature*, 367:576-78 (1993). These data have suggested that VEGFR-2 is a major regulator of vasculogenesis and angiogenesis. VEGF-C may thus have a unique effect in lymphatic endothelium and a more redundant function shared with VEGF in angiogenesis and possibly permeability regulation of several types of endothelia. Because VEGF-C stimulates the VEGFR-2 and promotes endothelial migration, a utility for VEGF-C is suggested as an inducer of angiogenesis of blood and lymphatic vessels in wound healing, tissue transplantation, in eye ^{diseases} ~~diseases~~, in the formation of collateral vessels to around arterial stenoses and into injured tissues after infarction.

Taken together, these results show an increased complexity of signalling in the vascular endothelium. They reinforce the concept that when organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases in several types of functionally and morphologically distinct vessels. However, upon suitable angiogenic stimuli, endothelial cells can re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. This process of angiogenesis concurrent with tissue development and regeneration depends on the tightly controlled balance between positive and negative signals for endothelial cell proliferation, migration, differentiation and survival.

Previously-identified growth factors promoting angiogenesis include the fibroblast growth factors, hepatocyte growth factor/scatter factor, PDGF and TGF- α . (See, e.g., Folkman, *Nature Med.* 1:27-31 (1995); Friesel and Maciag, *FASEB J.* 9:919-25 (1995); Mustonen and Alitalo, *J. Cell Biol.*, 129:895-98 (1995). However, VEGF has been the only growth factor relatively specific for endothelial cells. The newly identified factors VEGF-B

C Mutational analysis of the cysteine residues involved in the interchain disulfide bridges ^{has} have shown that, in contrast to PDGF, VEGF dimers need to be held together by these covalent interactions in order to maintain biological activity. Disulfide linking of the VEGF-C polypeptide chain was evident in the analysis of VEGF-C in nonreducing conditions.

C VEGFR-3, which thus distinguishes between VEGF and VEGF-C, is closely related ⁱⁿ to structure to VEGFR-1 and VEGFR-2. Finnerty, *et al.*, *Oncogene*, 8:2293-98 (1993); Galland, *et al.*, *Oncogene*, 8:1233-40 (1993); Pajusola, *et al.*, *Cancer Res.*, 52:5738-43 (1992). However, the mature form of VEGFR-3 differs from the two other VEGFRs in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola, *et al.*, *Oncogene*, 9:3545-55 (1994). Another difference is that the 4.5 and 5.8 kb VEGFR-3 mRNAs encode polypeptides differing in their C-termini and apparently in their signalling properties due to the use of alternative 3' exons. Borg *et al.*, *Oncogene*, 10:973-84 (1995); Pajusola *et al.*, *Oncogene*, 8:2931-37 (1993).

Besides VEGFR-3, VEGFR-2 tyrosine kinase also is activated in response to VEGF-C. VEGFR-2 mediated signals cause striking changes in the morphology, actin reorganization and membrane ruffling of porcine aortic endothelial cells overexpressing this receptor. In these cells, VEGFR-2 also mediated ligand-induced chemotaxis and mitogenicity. Waltenberger *et al.*, *J. Biol. Chem.*, 269:26988-95 (1994). Similarly, the receptor chimera CSF-1R/VEGFR-3 was mitogenic when ectopically expressed in NIH3T3 ^{3T3} fibroblastic cells, but not in porcine aortic endothelial cells (Pajusola *et al.*, 1994). Consistent with such results, the bovine capillary endothelial cells ^(BCE) which express VEGFR-2 mRNA but very little or no VEGFR-1 or VEGFR-3 mRNAs, showed enhanced migration when stimulated with VEGF-C. As shown here, light microscopy of the BCE cell cultures in collagen gel also suggested that VEGF-C stimulated the proliferation of these cells. The already existing data thus indicate that the VEGF ligands and receptors show a great specificity in their signalling, which may be cell type dependent.

The expression pattern of the VEGFR-3 (Kaipainen *et al.*, *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995)) suggests that VEGF-C may function in the formation of the venous and lymphatic vascular systems during embryogenesis. Constitutive expression of VEGF-C in adult tissues shown

latter. Proteolytic processing of the VEGF-C precursor may occur at more than one cleavage site because the 32 kD molecular mass of the recombinant secreted ligand was also less than the deduced molecular mass of VEGF-C ORF without the signal peptide. By extrapolation from studies of the structure of PDGF (Heldin, *et al.*, *Growth Factors*, 8:245-52 (1993)), one can speculate that the region critical for receptor binding and activation by VEGF-C is contained within the amino-terminal first 180 or so amino acid residues of the secreted VEGF-C protein lacking the signal sequence. In fact, the region critical for receptor binding and activation by VEGF-C is believed to be contained within the first approximately 120 amino acid residues of the secreted VEGF-C protein lacking the signal sequence. Thus, the 23 kD polypeptide binding VEGFR-3 is likely to represent the VEGF-homologous domain. After biosynthesis, the nascent VEGF-C polypeptide may be glycosylated at three putative N-linked glycosylation sites identified in the deduced VEGF-C amino acid sequence.

The carboxyl terminal amino acid sequences, which increase the length of the VEGF-C polypeptide in comparison with other ligands of this family, show a pattern of spacing of cysteine residues reminiscent of the ^{Bellanti} ~~Bellanti~~ ring 3 protein (BR3P) sequence (Dignam and Case, *Gene*, 88:133-40 (1990); Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990)). This novel C-terminal silk protein-like structural motif of VEGF-C may fold into an independent domain, which, on the basis of the considerations above, is at least partially cleaved off after biosynthesis. Interestingly, at least one cysteine motif of the BR3P type is also found in the carboxyl terminus of VEGF. In our experiments both the putative precursor and cleaved ligand were detected in the cell culture media, although processing was apparently cell-associated on the basis of the pulse-chase experiments. The determination of the amino terminal sequence of the isolated carboxyl terminal fragment will allow the identification of the proteolytic processing site. The generation of antibodies against different parts of the VEGF-C molecule will allow the exact determination of the precursor-product relationship and ratio, their cellular distribution, and the kinetics of processing and secretion.

VEGF-C has a conserved pattern of eight cysteine residues, which may participate in the formation of intra- and interchain disulfide bonds, creating an antiparallel dimeric biologically active molecule, similar to PDGF.

Ligands of the
C which are ligands for the Flt4 receptor tyrosine kinase (VEGFR-3). ~~Claimed~~
C ^{invention} ligands are members of a family of platelet-derived growth factors/vascular
endothelial growth factors which promote mitosis and proliferation of vascular
endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4
5 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma
cell line (ATCC CRL1435). When applied to a population of cells expressing
the Flt4 receptor, ligands of the invention stimulate autophosphorylation,
resulting in receptor activation. The invention also provides inhibitors of the
Flt4 receptor, including antibodies directed against the ligand. A ligand
10 according to the invention may be coexpressed as a larger precursor which is
cleaved to produce the ligand. A coexpressed region in some cases results
from alternative splicing of RNA of the ligand gene. Such a co-expressed
region may be a function of the particular expression system used to obtain the
ligand. The skilled artisan understands that in recombinant production of
15 proteins, additional sequence may be expressed along with a functional peptide
depending upon the particular recombinant construct used to express the
protein, and subsequently removed to obtain the desired ligand. In some cases
the recombinant ligand can be made lacking certain residues of the
endogenous/natural ligand. Moreover, it is well-known in that conservative
20 replacements may be made in a protein which do not alter the function of the
protein. Accordingly, it is anticipated that such alterations are within the
scope of the claims. It is intended that the precursor sequence shown in SEQ
ID NO: 33 is capable of stimulating the Flt4 ligand without any further
processing in a manner similar to that in which VEGF stimulates its receptor
25 in its unprocessed form.

Results reported herein show that VEGFR-3 transmits signals
for a novel growth factor. This conclusion is based on the specific binding of
VEGF-C to recombinant Flt4EC (Flt4 extracellular domain) protein and the
induction of VEGFR-3 autophosphorylation by medium from VEGF-C
30 transfected cells. In contrast, VEGF and PlGF did not show specific binding
to VEGFR-3 or induce its autophosphorylation.

A major part of the difference in the observed molecular mass
of the purified and recombinant VEGF-C and the deduced molecular mass of
the VEGF-C encoded by the VEGF-C open reading frame (ORF) may be due
35 to proteolytic removal of sequences in the carboxyl terminal region of the

Figure 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L (VEGF-C) expression vector.

C 5 Figure 12 shows Northern blotting analysis of Flt4-L (VEGF-C) mRNA in tumor cell lines ^{and in brain tissue}.

C Figure 13A is an autoradiograph showing recombinant ^{VEGF-C} ~~VEGF-C~~ isolated following a pulse-chase experiment and electrophoresed via SDS-PAGE under reducing conditions.

C 10 Figure 13B is a photograph of polyacrylamide gel showing that recombinant ^{VEGF-C} ~~VEGF-C~~ forms are disulfide-linked in nonreducing conditions.

Figure 14A and 14B depict Western blots showing that VEGF-C stimulates autophosphorylation of VEGFR-2 (KDR) but has no effect on PDGFR- β phosphorylation.

15 Figure 15A and 15B show that VEGF-C stimulates endothelial cell migration in a three-dimensional collagen gel assay.

Figure 16A shows the expression of VEGF-C mRNA in human adult tissues.

Figure 16B shows the expression of VEGF, VEGF-B, and VEGF-C in selected human fetal tissues.

20 Figure 17 schematically depicts the chromosomal localization of the VEGF-C gene.

Figure 18 is a Northern blot hybridization study showing the effects of hypoxia on the mRNA expression of VEGF-A, VEGF-B and VEGF-C.

25 **DETAILED DESCRIPTION OF THE INVENTION**

C Described herein is the isolation of a novel vascular endothelial growth factor ^{the cloning of a cDNA encoding this growth factor} and its cloning from a cDNA library prepared from the human prostatic adenocarcinoma cell line PC-3. The isolated cDNA encodes a protein which is proteolytically processed and secreted to cell culture medium. 30 The secreted protein, designated VEGF-C, binds to the extracellular domain of Flt4 (designated VEGFR-3) and induces tyrosine autophosphorylation of Flt4 and VEGFR-2. VEGF-C also stimulates the migration of endothelial cells in collagen gel.

The present invention also is directed to novel growth factors

oligonucleotides, and peptides which block the Flt4 receptor, all of which are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a schematic diagram showing major endothelial cell
5 receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

Figure 2 schematically depicts the construction of the pLTRFlt4l expression vector.

Figure 3 schematically depicts the construction of the
10 baculovirus vector encoding a secreted soluble Flt4 extracellular domain (Flt4EC).

Figure 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

C *Figures 5A, 5B, and 5C show*
15 *that the major tyrosyl phosphorylated*
polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide (VEGFR-3).

Figure 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.

C *Chromatographic fractions from*
C *No affinity purification*
20 *the Western analysis of Flt4 ligand (VEGF-C) isolated from PC-3 conditioned medium.*

Figure 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand (VEGF-C), VEGF, or PlGF.

25 Figure 9A schematically depicts the cloning and analysis of the Flt4 ligand, VEGF-C. The VEGF-C coding sequence (shaded boxes) and signal sequence (ss) are depicted between 5' and 3' untranslated (ut) nucleic acid regions.

C
30 Figure 9B shows the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA. The cleavage site for the putative signal peptide is indicated with a shaded triangle.

Figure 10 shows a comparison of the deduced amino acid sequences of PDGF-A, -B, two PlGF isoforms, four VEGF isoforms and Flt4 ligand (VEGF-C).

sub
C

In another aspect, the invention includes an antibody which is specifically reactive with polypeptides of the invention. Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells, or they may be used to block or activate the Flt4 receptor.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*.

Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, echogenic, or radioactive agent for imaging.

Other, non-radioactive labels, such as biotin and avidin, may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors are used to accelerate angiogenesis, *e.g.*, during wound healing, or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands also may be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using detectably-labeled ligand. An Flt4 ligand according to the invention also may be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention also may be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation, and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomias. For example, such inhibitors may be used to arrest metastatic growth or spread, or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense

comprises approximately amino acids 1-120 of SEQ ID NO: 33. Another preferred polypeptide of the invention comprises approximately amino acids 1-180 of SEQ ID NO: 33.

The present invention also provides a cDNA encoding a novel
5 polypeptide, designated VEGF-C, that is structurally homologous to VEGF. VEGF-C is a ligand for the FLT4 receptor tyrosine kinase (VEGFR-3), a receptor tyrosine kinase related to VEGFR-1 and VEGFR-2 that does not bind VEGF. VEGFR-3 is expressed in venous and lymphatic endothelia of fetal tissues and predominantly in lymphatic endothelial of adult tissues. Kaipainen
10 et al., *Cancer Res.*, 54:6571-77 (1994); Kaipainen et al., *Proc. Natl. Acad. Sci. USA*, 92:3566-70 (1995).

Thus, in a preferred embodiment, the invention includes a purified and isolated nucleic acid (e.g., a DNA or an RNA) encoding an Flt4 ligand precursor. Due to the degeneracy of the genetic code, numerous such
15 coding sequences are possible, each having in common the coding of the amino acid sequence shown in SEQ ID NO: 33. As set forth above, the invention includes polypeptides which comprise a portion of the amino acid sequence shown in SEQ ID NO: 33 and which bind the Flt4 receptor tyrosine kinase (herein designated VEGFR-3); the invention also is intended to include
20 nucleic acids encoding these polypeptides. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase (VEGFR-3). The nucleotide sequence shown in SEQ ID NO:32 contains a preferred nucleotide sequence encoding the Flt4 ligand (VEGF-C).

25 The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising a DNA encoding the Flt4 ligand, and host cells comprising the vectors. Preferred vectors of the invention are capable of expressing the Flt4 ligand under the control of
30 appropriate promoters and other control sequences. A preferred vector of the invention is plasmid pFLT4-L, having ATCC accession no. ⁷⁷²³¹97321.
C

The invention further includes a method of making polypeptides
of the invention. In a preferred method, a nucleic acid or vector of the invention is expressed in a host cell, and a polypeptide of the invention is
35 purified from the host cell or the host cell growth medium.

and VEGFR-1 also binds the related placenta growth factor (PlGF; Kd about 200 pM), while the ligands for Tie, Tek, and Flt4 have not yet been reported.

SUMMARY OF THE INVENTION

5 The present invention provides a ligand for the Flt4 receptor tyrosine kinase. Thus, the invention provides a purified and isolated polypeptide which specifically binds to the Flt4 receptor tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

10 The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in SEQ ID NO: 33. Thus, the invention includes a purified and isolated polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

C. A putative ³³ amino acid signal peptide has been identified in
15 the amino acid sequence shown in SEQ ID NO: 33. Thus, in a related aspect, the invention includes a purified and isolated polypeptide comprising amino acids 1-³¹⁷ of SEQ ID NO: 33. The Flt4 ligand precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand (herein designated VEGF-C). Thus, the
C 20 invention includes a polypeptide having an amino acid sequence comprising a portion of SEQ ID NO: ³³3, the portion encoding a fragment capable of specifically binding to Flt4. A preferred fragment has a molecular weight of about 23 kDa as assessed by SDS-PAGE under reducing conditions. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the
25 cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing conditions.

Evidence suggests that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage to produce a mature,
30 naturally-occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. Accordingly, preferred ^{polypeptides} polypeptides of the invention include polypeptides comprising amino acids 1-120, 1-121, 1-122, 1-123, 1-124 ... 1-178, 1-179, and 1-180 of SEQ ID NO: 33, wherein said polypeptides specifically bind to an Flt4 receptor tyrosine kinase. A preferred Flt4 ligand

rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only
5 endothelial cells have been reported to proliferate in response to VEGF, and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase (VEGFR-3) is closely related
10 in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola *et al.*, *Cancer Res.*, 52:5738-5743 (1992). The
C 4,5 and 5.8 kb ^{Flt4} ~~Flt4~~ mRNAs encode polypeptides which differ in their C-
15 termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by *in situ* hybridization in the angioblasts of head mesenchyme, the cardinal vein,
20 and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In
C 12.5 day p.c. embryos the ^{Flt4} ~~Flt4~~ signal is observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to
25 developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

Five endothelial cell specific receptor tyrosine kinases, Flt-1
30 (VEGFR-1), KDR/Flk-1 (VEGFR-2), Flt4, Tie and Tek/Tie-2 have so far been described, which possess the intrinsic tyrosine kinase activity essential for signal transduction. Targeted mutations inactivating Flt-1, Flk-1, Tie and Tek in mouse embryos have indicated their essential and specific roles in
35 vasculogenesis and angiogenesis at the molecular level. VEGFR-1 and VEGFR-2 bind VEGF with high affinity (Kd 16 pM and 760 pM, respectively)

is a dimeric glycoprotein of disulfide-linked 23 kDa subunits. Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration *in vitro*. Four VEGF isoforms, encoded by distinct mRNA splice variants, appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each isoform has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues remain cell surface associated and have a strong affinity for heparin.

VEGF was originally purified from several sources on the basis of its mitogenic activity toward endothelial cells, and also by its ability to induce microvascular permeability, hence it is also called vascular permeability factor (VPF). VEGF produces signals through two receptor tyrosine kinases, VEGFR-1 (FLT-1) and VEGFR-2 (KDR/Flk-1), which are expressed specifically on endothelial cells. The VEGF-related placenta growth factor (PlGF) was recently shown to bind to VEGFR-1 with high affinity. PlGF was able to enhance the growth factor activity of VEGF, but it did not stimulate endothelial cells on its own. Naturally occurring VEGF/PlGF heterodimers were nearly as potent mitogens as VEGF homodimers for endothelial cells.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm produces VEGF at the capillary ingrowth stage. See Breier, *et al.*, *Development*, 114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors
5 are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, *et al.*, *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in Figure 1.

10 Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, *et al.*,
15 *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of
20 endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dimerizing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the platelet
25 derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, *et al.*, *Growth Factors*, 7:261-266 (1992). Transforming growth factor α (TGF α) is an angiogenic factor secreted by several tumor cell types and by macrophages. Hepatocyte growth factor (HGF), the ligand of the *c-met* proto-oncogene-encoded receptor, also is
30 strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain differentiated
35 functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor



- 1 -

RECEPTOR LIGAND

This is a continuation-in-part of United States Patent Application
Serial Number 08/510,133, filed August 1, 1995, ~~DI~~

FIELD OF THE INVENTION

5 The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

BACKGROUND OF THE INVENTION

10 Developmental growth, the remodelling and regeneration of adult tissues, as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (*in situ*) differentiating endothelial cells is termed
15 vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, *et al.*, *Devel. Biol.*, 125:441-450 (1988).

20 Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their
25 tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, *et al.*, *Microvasc. Rev.*, 14:51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation,
30 migration, differentiation, and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most significantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, *et al.*, *J. Biol. Chem.*, 267:10931-10934 (1992).

08/585 895



- 52 -

ABSTRACT

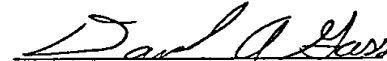
Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.

JOINT INVENTORS

"EXPRESS MAIL" mailing label No.
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being
deposited with the United States Postal Service
"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR §1.10 on the date
indicated above and is addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231


David A. Gass

**APPLICATION FOR
UNITED STATES LETTERS PATENT**

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo, a citizen of Finland, residing at
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a
new and useful "RECEPTOR LIGAND", of which the following is a specification.

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:



David A. Gass
Reg. No: 38,153

January 12, 1996

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

| CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY) | | | | | | |
|---|-----------|-----------|--------------|----------|---------------------------|----------|
| | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
| | NO. FILED | NO. EXTRA | RATE | FEE | RATE | FEE |
| BASIC FEE | | | | \$375.00 | | \$750.00 |
| TOTAL | 16 - 20 | = 0 | X 11 = | \$ | X 22 = | \$ |
| INDEP. | 3 - 3 | = 0 | X 39 = | \$ | X 78 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | + 125 = | \$ | + 250 = | \$ |
| Filing Fee: | | | | \$ | OR | \$750.00 |

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ _____

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ _____

D. Other Fees

☐ Recording Assignment [Fee -- \$40.00 per assignment] \$ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached [Fee -- \$130.00] \$ _____

☐ Other \$ _____

Total Fees Enclosed **\$750.00**

6. Method of Payment of Fees

☒ Check in the amount of:

\$750.00

☐ Charge Deposit Account No. 13-2855 in the amount of:
A copy of this Transmittal is enclosed.

\$ _____

☐ Not enclosed

3. Declaration or Oath

- ☐ Enclosed
 - ☐ Executed by (check all applicable boxes)
 - ☐ Inventor(s)
 - ☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)
 - ☐ Joint inventor or person showing a proprietary interest on behalf of
inventor who refused to sign or cannot be reached
 - ☐ The petition required by 37 CFR 1.47 and the statement required
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer-readable copy of sequence listing containing nucleotide and/or amino
acid sequence
- ☒ Statement pursuant to 37 C.F.R. §1.821(f)
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

| COUNTRY | APPLICATION NO. | FILED |
|---------|-----------------|-------|
| | | |
| | | |
| | | |

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other



00 585 19

PATENT APPLICATION**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Docket No: 28113/33072

PATENT APPLICATION TRANSMITTAL

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): 1-00 374
Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

1. Type of Application

This new application is for a


- ☒ utility patent.
☐ design patent.

2. Application Papers Enclosed

- 1 Title Page
49 Pages of Specification (excluding Claims, Abstract & Drawings)
2 Pages of Claims
1 Page of Abstract
24 Sheets of Drawings (Figs. 1 to 18)
☐ Formal
1 ☒ Informal

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.


David A. Gass

PATENT APPLICATION SERIAL NO. 585895

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET



4130 105

0250 Trans #2 1/2

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:) I hereby certify that this paper is being deposited
Alitalo et al.) with the United States Postal Service as first
Serial No.: 08/585,895) class mail, postage prepaid, in an envelope
Filed: January 12, 1996) addressed to: Assistant Commissioner for
For: Receptor Ligand) Patents, Washington, D.C. 20231, on this date:
Group Art Unit: Not yet assigned) Dated: March 28, 1996
Examiner: Not yet assigned) David A. Gass
) David A. Gass
) Registration No. 38,153
) Attorney for Applicant(s)

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

March 28, 1996

By: David A. Gass

David A. Gass
Reg. No: 38,153



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| In the Application of: |) | I hereby certify that this paper is being deposited |
| Alitalo et al. |) | with the United States Postal Service as first |
| Serial No.: 08/585,895 |) | class mail, postage prepaid, in an envelope |
| Filed: January 12, 1996 |) | addressed to: Assistant Commissioner for |
| For: Receptor Ligand |) | Patents, Washington, D.C. 20231, on this date: |
| Group Art Unit: Not yet assigned |) | Dated: <u>March 28, 1996</u> |
| Examiner: Not yet assigned |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| |) | Registration No. 38,153 |
| |) | Attorney for Applicant(s) |

TRANSMITTAL OF EXECUTED DECLARATION

Assistant Commissioner for Patents
Washington, D.C. 20231

Attention: Application Branch

Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application. No Notice to File Missing Parts has been received by the Applicants.

Also enclosed is a check in the amount of \$130.00 in payment of the fee for submission of the declaration. See 37 C.F.R. §1.16(e).

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

March 28, 1996

By: David A. Gass
David A. Gass
Reg. No: 38,153

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed on January 12, 1996, as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ Yes ☐ No

| (Application Serial Number) | (Country) | (Day/Month/Year Filed) |
|-----------------------------|-----------|------------------------|
| | | |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| (Application Serial Number) | (Day/Month/Year Filed) |
|-----------------------------|------------------------|
| | |

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

| (Application Serial Number) | (Day/Month/Year Filed) | (Status-Patented, Pending or Abandoned) |
|-----------------------------|------------------------|---|
| 08/510,133 | 01 August 1995 | Pending |
| | | |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

| | | | |
|----------------------------|-------------------------------|------------------------------|---------------------------------|
| Alvin D. Shulman (19,412) | Trevor B. Joike (25,542) | Richard A. Schurr (30,890) | James J. Napoli (32,361) |
| Donald J. Broth (19,490) | Timothy J. Vezau (26,348) | Anthony Nimmo (30,920) | Richard M. La Barge (32,254) |
| Owen J. Murray (22,111) | Carl E. Moore, Jr. (26,487) | Christine A. Dudzik (31,245) | Jeffrey W. Smith (33,455) |
| Allen H. Gerstein (22,218) | Richard H. Anderson (26,526) | Kevin D. Hogg (31,839) | Douglas C. Hochstetler (33,710) |
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| Michael F. Borun (25,447) | William E. McCracken (30,195) | Martin J. Hirsch (32,237) | David A. Gass (38,153) |

Send correspondence to: David A. Gass

| FIRM NAME | PHONE NO. | STREET | CITY & STATE | ZIP CODE |
|--|--------------|--|-------------------|------------|
| Marshall, O'Toole, Gerstein, Murray & Borun | 312-474-6300 | 6300 Sears Tower 233 South Wacker Drive | Chicago, Illinois | 60606-6402 |

| | |
|---|--------------------------------------|
| Full Name of First or Sole Inventor Kari Alitalo | Citizenship Finland |
| Residence Address - Street Nyyrikintie 4A | Post Office Address - Street Same |
| City (Zip) 02100 Espoo | City (Zip) Same |
| State or Country FINLAND | State or Country Same |
| Date March 14, 1996 | Signature [Signature] |

See second page for additional inventor

See reverse for relevant rules & statutes

| | |
|---|---|
| Second Joint Inventor, if any Vladimir Joukov | Citizenship Finland <i>Russia</i> |
| Residence Address - Street Topeliuksenkatu 32G8 | Post Office Address - Street Same |
| City (Zip) 00290 Helsinki <i>FI</i> | City (Zip) Same |
| State or Country FINLAND | State or Country Same |
| Date <input checked="" type="checkbox"/> <i>March 14, 1996</i> | Signature <input checked="" type="checkbox"/> <i>V. Joukov</i> |



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|--------------------|-------------|-----------------------|------------------------|
| 08/585,298 | 01/11/96 | DAVID A. GASS | 081107/00072 |

0272/0913
DAVID A. GASS
MARSHALL S. TOOLE, GERSTEIN MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

DATE MAILED: 05/13/96

NOTICE TO FILE MISSING PARTS OF APPLICATION FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 150.00 for large entities or \$ 15.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 150.00.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
_____. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.





#130

122

0300
5/16/96PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| Applicants: Alitalo et al. |) | "EXPRESS MAIL" |
| Serial No: 08/585,895 |) | Mailing label No. EM118660766US |
| Filed: January 16, 1996 |) | Date of Deposit: |
| Title: RECEPTOR LIGAND |) | May 24, 1996 |
| Group Art Unit: Not yet assigned |) | I hereby certify that this paper (or fee) |
| Examiner: Not yet assigned |) | is being deposited with the United |
| |) | States Postal Service "EXPRESS |
| |) | MAIL POST OFFICE TO ADDRESSEE" |
| |) | service under 37 CFR §1.10 on the |
| |) | date indicated above and is addressed |
| |) | to the Assistant Commissioner for |
| |) | Patents, |
| |) | Washington, D.C., 20231. |
| |) | <i>Mark Bonadonna</i> |
| |) | Mark Bonadonna |

Petition to Accord a Filing Date of January 12, 1996,
Pursuant to 37 C.F.R. §§ 1.6, 1.10,
and 1.53, and M.P.E.P. §506.02

or, in the alternative,

Petition to Suspend Rules Pursuant to
35 U.S.C. §21 and 37 C.F.R. § 1.183
to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Special Program Law Office

Dear Sir:

The Applicants request that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. The application was filed in accordance with 37 C.F.R. §1.10 and 1.53(a) on January 12, 1996.

If this request is denied, then the Applicants hereby petition to accord a filing date of January 12, 1996, pursuant to 37 C.F.R. §§ 1.6, 1.10, and 1.53, and M.P.E.P. §506.02. In the alternative, the Applicants petition to suspend the rules pursuant to 35 U.S.C. §21 and 37 C.F.R. §1.183, to Accord Filing Date of January 12, 1996.

I. Petition for Review of Refusal to Accord Filing Date Pursuant to 37 C.F.R. §§ 1.6, 1.10, 1.17(h) and 1.53, and M.P.E.P. §506.02

A. Statement of Facts

The above-identified patent application was filed in accordance with 37 C.F.R. §1.53(a) on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10. Copies of the Applicants' transmittal letter, specification cover sheet, and express mail mailing receipt are submitted herewith as Exhibits 1, 2, and 3, respectively. The transmittal letter and specification cover sheet contain certificates of mailing in accordance with 37 C.F.R. §1.10, dated January 12, 1996.

On May 15, 1996, the Patent and Trademark Office mailed a Notice to File Missing Parts of Application -- Filing Date Granted. (Exhibit 4.) However, the filing date on the Notice was Tuesday, January 16, 1996, instead of January 12, 1996.

B. Argument

The Patent and Trademark Office has not identified any defects in the application or certificates of mailing dated Friday, January 12, 1996. Friday, January 12, 1996, was not a Saturday, Sunday, or Federal holiday.¹ Accordingly, under the rules promulgated by the Commissioner, the above-identified application properly should be considered as having been filed on January 12, 1996. See 37 C.F.R. §1.10 (a). Correction of the filing date to January 12, 1996, is respectfully requested.

II. Conditional Petition to Suspend Rules to Accord a Filing Date of January 12, 1996

If the foregoing petition is denied on the grounds that the Commissioner declared January 12, 1996, to be a "Federal holiday" as that term is used in 37 C.F.R. §1.10, then the Applicants hereby petition the Commissioner to suspend the rules pursuant to 37 C.F.R. §1.183, and to accord the present application a filing date of January 12, 1996. This petition has been filed following a telephone interview between the undersigned attorney and Examiner

¹ As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

Nguyen concerning this matter on May 20, 1996, which interview the Applicants acknowledge with thanks.

A. Statement of Facts

The Applicants, through their attorneys at Marshall, O'Toole, Gerstein, Murray & Borun, prepared the above-identified application for filing on or before January 12, 1996, on the informed belief that a manuscript (authored by the Applicants and others) describing aspects of the invention would be published in *The EMBO Journal*, Volume 15, Number 2, on January 15, 1996 (Martin Luther King's Birthday, a Federal Holiday). The Application was filed on Friday, January 12, 1996, using the "Express Mail" procedures set forth in 37 C.F.R. §1.10, as explained in detail in Section I above. (See Exhibits 1-3.) However, the application was accorded a filing date of January 16, 1996. (See Exhibit 4.)

The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

B. Argument

The Applicants' reliance on the Express Mail procedures of 37 C.F.R. §1.10 for the present application, to obtain a filing date of January 12, 1996, was reasonable, because January 12, 1996, was not a Saturday, Sunday, or recognized Federal holiday. If January 12 was deemed a "Federal holiday" by the Commissioner, it was so deemed, without advance warning to the Applicants, due to an unscheduled and unforeseeable event -- adverse weather conditions in the District of Columbia. An apparent purpose of deeming such days to be "Federal holidays" is to protect applicants' patent rights, by allowing for the timely filing of papers or fees on the next succeeding business day. See M.P.E.P. §510. However, the effect of denying the present Applicants a filing date of January 12, 1996, on the grounds of an unscheduled, weather-related "Federal holiday" being declared, may be to destroy the present Applicants' valuable patent rights in foreign countries. The Applicants submit that the foregoing unforeseeable circumstances comprise an extraordinary situation, and that justice requires the suspension of rules to accord the present application a filing date of January 12,

1996, to preserve the Applicants' foreign patent rights. The granting of such a filing date is not believed to contravene any requirements of the patent statutes. In fact, the granting of the January 12, 1996, filing date is submitted to be in complete harmony with the purpose and intent of 35 U.S.C. §21 and 37 C.F.R. §§1.6 and 1.10.

SUMMARY

The Applicants respectfully request and petition that the present application be accorded a filing date of January 12, 1996. The present petition is accompanied by a check for \$130.00 in payment of the petition fee set forth in 37 C.F.R. §1.17(h). The Commissioner is authorized to charge any necessary additional fees due in connection with this petition to deposit account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

Dated: MAY 24, 1996



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN, .
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| Applicants: Alitalo et al. |) | "EXPRESS MAIL" |
| |) | Mailing label No. EM118660766US |
| Serial No: 08/585,895 |) | |
| Filed: January 16, 1996 |) | Date of Deposit: |
| |) | May 24, 1996 |
| Title: RECEPTOR LIGAND |) | |
| Group Art Unit: Not yet assigned |) | I hereby certify that this paper (or fee) |
| Examiner: Not yet assigned |) | is being deposited with the United |
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| |) | service under 37 CFR §1.10 on the |
| |) | date indicated above and is addressed |
| |) | to the Assistant Commissioner for |
| |) | Patents, |
| |) | Washington, D.C., 20231. |
| |) | |
| |) | <i>Mark Bonadonna</i> |
| |) | Mark Bonadonna |

Petition to Accord a Filing Date of January 12, 1996,
Pursuant to 37 C.F.R. §§ 1.6, 1.10,
and 1.53, and M.P.E.P. §506.02

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to Accord Filing Date of January 12, 1996

Assistant Commissioner for Patents
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Attn: Special Program Law Office

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¹ As set forth in M.P.E.P. §710.05, the Federal holidays are New Year Day, Martin Luther King's Birthday, Washington's Birthday, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving Day, Christmas Day, and Inauguration Day.

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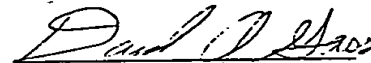
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SUMMARY

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Respectfully submitted,

Dated: MAY 24, 1996



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



Exhibit 1

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No: 28113/33072

PATENT APPLICATION TRANSMITTAL

*Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladimir Joukov

Title: "Receptor Ligand"

1. Type of Application

This new application is for a

- ☒ utility patent.
☐ design patent.

2. Application Papers Enclosed

- | | |
|-------------------------------------|--|
| 1 | Title Page |
| 49 | Pages of Specification (excluding Claims, Abstract & Drawings) |
| 2 | Pages of Claims |
| 1 | Page of Abstract |
| 24 | Sheets of Drawings (Figs. 1 to 18) |
| <input type="checkbox"/> | Formal |
| <input checked="" type="checkbox"/> | Informal |

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on January 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473137204US.


David A. Gass

3. Declaration or Oath

☐ Enclosed

☐ Executed by (check all applicable boxes)

☐ Inventor(s)

☐ Legal representative of inventor(s)
(37 CFR 1.42 or 1.43)

☐ Joint inventor or person showing a proprietary interest on behalf of
inventor who refused to sign or cannot be reached

☐ The petition required by 37 CFR 1.47 and the statement required
by 37 CFR 1.47 are enclosed. See Item 5D below for fee.

☒ Not enclosed - the undersigned attorney or agent is authorized to file this
application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

☐ Preliminary Amendment

☐ Information Disclosure Statement

☐ Declaration of Biological Deposit

☒ Computer-readable copy of sequence listing containing nucleotide and/or amino
acid sequence

☒ Statement pursuant to 37 C.F.R. §1.821(f)

☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27

☐ Associate Power of Attorney

☐ Verified translation of a non-English patent application

☐ An assignment of the invention

☐ Certified copy(ies) of application(s):

| COUNTRY | APPLICATION NO. | FILED |
|---------|-----------------|-------|
| | | |
| | | |
| | | |

from which priority under 35 USC 119 is claimed ☐ is(are) attached.

☐ will follow.

☐ Other

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

| CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY) | | | | | | |
|---|-----------|-----------|--------------|----------|---------------------------|----------|
| | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
| | NO. FILED | NO. EXTRA | RATE | FEE | RATE | FEE |
| BASIC FEE | | | | \$375.00 | | \$750.00 |
| TOTAL | 16 - 20 | = 0 | X 11 = | \$ | X 22 = | \$ |
| INDEP. | 3 - 3 | = 0 | X 39 = | \$ | X 78 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | + 125 = | \$ | + 250 = | |
| Filing Fee: | | | | \$ | OR | \$750.00 |

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ _____

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ _____

D. Other Fees

☐ Recording Assignment (Fee -- \$40.00 per assignment) \$ _____

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached (Fee -- \$130.00) \$ _____

☐ Other \$ _____

Total Fees Enclosed \$750.00

6. Method of Payment of Fees

☒ Check in the amount of: \$750.00

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

☐ Not enclosed

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to David A. Gass at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

January 12, 1996



JOINT INVENTORS


Exhibit 2

"EXPRESS MAIL" mailing label No.
EG473137204US.

Date of Deposit: January 12, 1996

I hereby certify that this paper (or fee) is being
deposited with the United States Postal Service

"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
service under 37 CFR §1.10 on the date
indicated above and is addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231


David A. Gass

APPLICATION FOR
UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo, a citizen of Finland, residing at
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov, a citizen of Finland,
residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have invented a
new and useful "RECEPTOR LIGAND", of which the following is a specification:



Exhibit 3

POST OFFICE TO ADDRESSEE **EXPRESS MAIL**
EMS

EG473137204US

ORIGIN (POSTAL USE ONLY)

| | | |
|-----------------|--------------------------|----------------------|
| DATE OF POSTAGE | DATE OF DELIVERY | POSTAGE |
| May 24 1996 | May 24 1996 | \$1.00 |
| Time to | Time to | |
| AM | PM | |
| Weight | Int'l Alpha Country Code | |
| lb | oz | |
| No Delivery | Acceptance | |
| Weekend | Check Initials | |
| | | Total Postage & Fees |
| | | \$1.50 |

SEE REVERSE SIDE FOR THE
SERVICE GUARANTEE AND LIMITS
ON THE INSURANCE COVERAGE

CUSTOMER USE ONLY

METHOD OF PAYMENT:

Express Mail Corporate Acct. No.:

Federal Agency Acct. No. or

Postal Service Acct. No.:

FROM: (PLEASE PRINT)

PHONE

28113/38072

DAVID A. GASS
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402

TO: (PLEASE PRINT)

PHONE

Assistant Commissioner
for Patents
Washington, D.C. 20231

Box Patent Application

EL 11-8 11/93

For Pickup or Tracking Call 1-800-222-1811

CUSTOMER COPY



Exhibit 4
UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|--------------------|-------------|-----------------------|------------------------|
| 08/535,895 | 01/16/96 | ALITALO | K 28113/33072 |

0272/0515
DAVID A GASS
MARSHALL O'TOOLE GERSTEIN MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

RECEIVED
MAY 20 1996

6-15-96 MARSHALL O'TOOLE

DATE MAILED: 05/15/96

**NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED**

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130.00 for large entities or \$ 65.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

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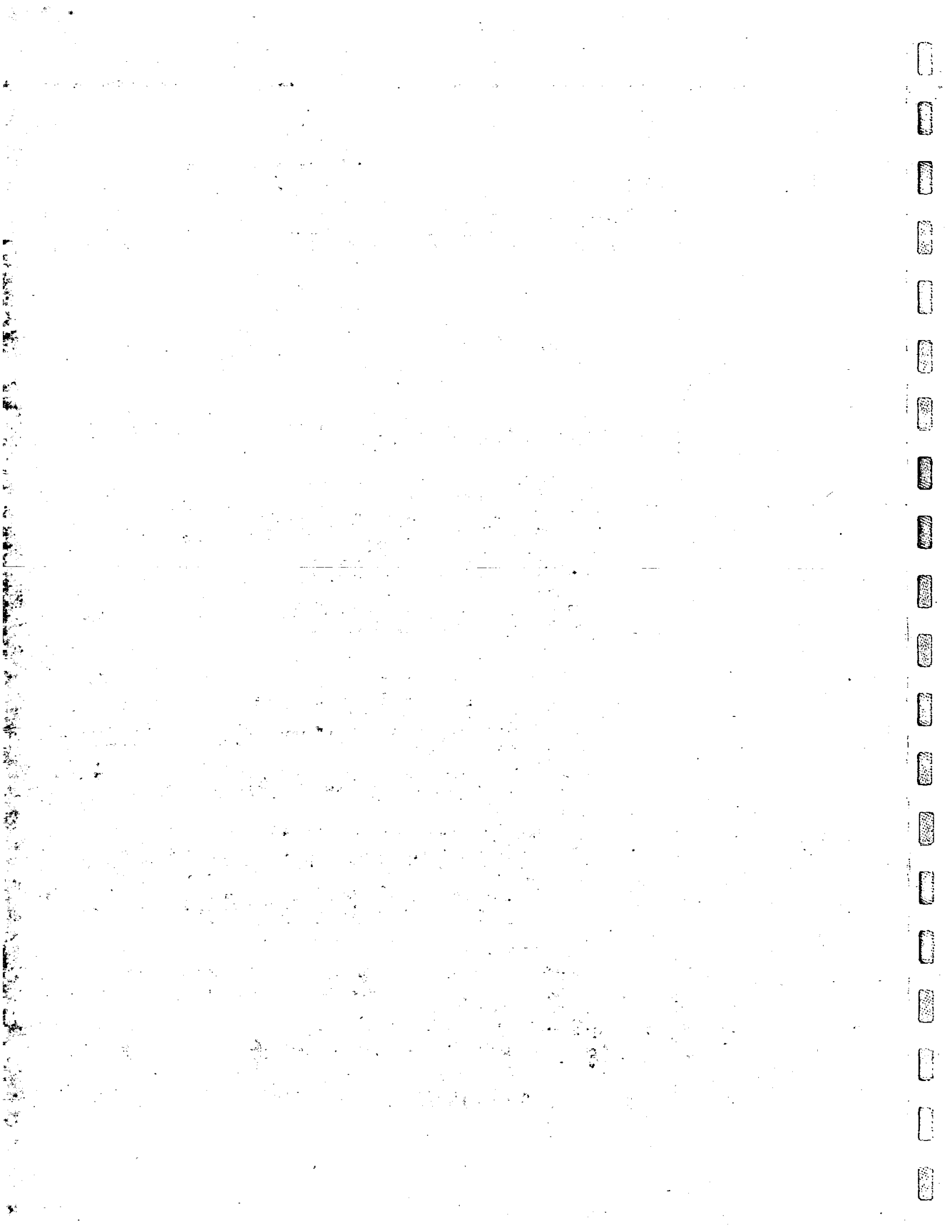
1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ _____ to complete the basic filing fee.
2. ☐ Additional claim fees of \$ _____ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
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☒ is missing.
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:
_____. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
10. ☐ The application does not comply with the Sequence Rules. See attached Notices to Comply with Sequence Rules 37 CFR 1.821-1.825.
11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

A copy of this notice MUST be returned with the response.

ATTORNEY'S/APPLICANTS COPY





130-122 B

PATENT # K
28113/33072
NOTE PA
NUMBER
FIVE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| Applicants: Alitalo et al. |) | "EXPRESS MAIL" |
| Serial No: 08/585,895 |) | Mailing label No.: EM118663762US |
| Filed: January 16, 1996 |) | Date of Deposit: |
| |) | June 25, 1996 |
| Title: RECEPTOR LIGAND |) | I hereby certify that this paper (or fee) |
| Group Art Unit: Not yet assigned |) | is being deposited with the United |
| Examiner: Not yet assigned |) | States Postal Service "EXPRESS |
| |) | MAIL POST OFFICE TO ADDRESSEE" |
| |) | service under 37 CFR §1.10 on the |
| |) | date indicated above and is addressed |
| |) | to the Assistant Commissioner for |
| |) | Patents, |
| |) | Washington, D.C., 20231. |
| |) | <u>Mark Bonadonna</u> |
| |) | Mark Bonadonna |

Petition to Expedite Handling of Earlier Filed Petition
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents
Washington, D.C. 20231

Attn: Petitions Office

Dear Sir:

On May 24, 1996, the Applicants filed a petition (the "Filing Date Petition") requesting that the above-identified patent application, which has been accorded a filing date of January 16, 1996, be accorded an earlier filing date of January 12, 1996. For the reasons set forth below, the Applicants request that the Filing Date Petition receive expedited handling. This request is accompanied by a check for \$130 in payment of the petition fee.

The Applicants request expedited handling for the purposes of international filings which will claim priority from the present application.

A. Statement of Facts

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The Applicants intend to file at least one application directed to the subject matter of the present application in a foreign country, claiming the priority benefit of the present application under the Paris Convention. Most foreign countries are "absolute novelty" countries wherein a publication of an invention that is available to the public on January 15, 1996, will bar patent protection to the invention based on an application having a priority date of January 16, 1996.

The present application is a continuation-in-part of U.S. Patent Application Serial No. 08/510,133, filed August 1, 1995. To obtain the priority benefit of both the parent application and the present application under the Paris Convention, the Applicants intend to file at least one foreign application on or before August 1, 1996.

B. Argument

The Applicants request an expedited decision on the Filing Date Petition to permit the Applicants to make an informed and accurate foreign filing that claims priority from the above-identified application. Specifically, the Applicants request that the Patent Office render its decision on the Filing Date Petition substantially in advance of the August 1, 1996.

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on the Filing Date Petition in advance of the August 1, 1996, Paris Convention deadline will permit the Applicants to identify this priority application by its official filing date.

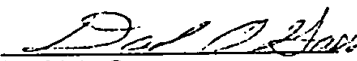
Second, a decision on the Filing Date Petition, substantially in advance of the August 1, 1996, treaty deadline, will permit the Applicants to evaluate and properly claim the subject matter of their invention in foreign applications. As explained in the Filing Date petition, the effect of denying the present Applicants a filing date of January 12, 1996, may be to destroy the present Applicants' valuable patent rights in foreign countries. A decision on the Filing Date Petition substantially in advance of the August 1, 1996, deadline will permit the Applicants to properly evaluate the value of the present application as a priority document under the patent laws of "absolute novelty" countries.

SUMMARY

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Respectfully submitted,

Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| Applicants: Alitalo et al. |) | "EXPRESS MAIL" |
| Serial No: 08/585,895 |) | Mailing label No.: EM118663762US |
| Filed: January 16, 1996 |) | Date of Deposit: |
| Title: RECEPTOR LIGAND |) | June 25, 1996 |
| Group Art Unit: Not yet assigned |) | I hereby certify that this paper (or fee) |
| Examiner: Not yet assigned |) | is being deposited with the United |
| |) | States Postal Service "EXPRESS |
| |) | MAIL POST OFFICE TO ADDRESSEE" |
| |) | service under 37 CFR §1.10 on the |
| |) | date indicated above and is addressed |
| |) | to the Assistant Commissioner for |
| |) | Patents, |
| |) | Washington, D.C., 20231. |
| |) | <i>Mark Bonadonna</i> |
| |) | Mark Bonadonna |

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Assistant Commissioner for Patents
Washington, D.C. 20231

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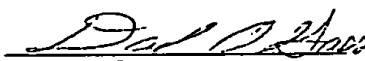
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Respectfully submitted,

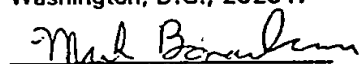
Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---|
| Applicants: Alitalo et al. |) | "EXPRESS MAIL" |
| |) | Mailing label No.: EM118663762US |
| Serial No: 08/585,895 |) | |
| |) | Date of Deposit: |
| Filed: January 16, 1996 |) | June 25, 1996 |
| |) | |
| Title: RECEPTOR LIGAND |) | I hereby certify that this paper (or fee) |
| |) | is being deposited with the United |
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| |) | Patents, |
| |) | Washington, D.C., 20231. |
| |) |  |
| |) | Mark Bonadonna |

Petition to Expedite Handling of Earlier Filed Petition
Pursuant to 37 C.F.R. §§ 1.181-183

Assistant Commissioner for Patents
Washington, D.C. 20231

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JUL 17 1996

PETITION OFFICE

Attn: Petitions Office

Dear Sir:

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Respectfully submitted,

Dated: 25 June 1996


David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

*** ACTIVITY REPORT ***

RECEPTION OK

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| USAGE TIME | 01'16 | |
| PAGES | 4 | |
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Jul. 17, 1996 9:54AM MARSHALL, O'TOOLE

No. 1196 P. 1/4
From: 0808

MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN

RICHARD H. ANDERSON
MICHAEL F. BORUN
DONALD J. BROTT
MADELINE MENICKS DEVEREUX
CHRISTINE A. DUDZIK
PATRICK D. ERTEL
ALLEN H. GERSTEIN
ROBERT M. GERSTEIN
MARTIN J. HIRSCH
DOUGLASS C. HOCHSTETLER
KEVIN D. HOGG
TREVOR B. JOIKE
RICHARD M. L. BARGE
WILLIAM E. McCracken
CARL E. MOORE, JR.
OWEN J. MURRAY
JAMES J. NAPOLI, Ph.D.
ANTHONY NABAO
EDWARD M. O'TOOLE
DONALD J. POCHOPIN, Ph.D.
NATE F. SCARFELLI
CYNTHIA L. SCHALLER
RICHARD A. SCHUMER
JEFFREY S. SHARP
ALVIN D. SHULMAN
JEFFRY W. SMITH
TIMOTHY J. VEZEAU
KARL A. VICK
JAMES P. ZELLEN

ATTORNEYS AT LAW
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606-6402
(312) 474-6300
FAX: (312) 474-0448

G. CHRISTOPHER BRAIDWOOD
RICHARD A. BRANDON
JANE J. CHOI
DAVID W. CLOUGH, Ph.D.
DAVID A. GASS
SCOTT M. GETTLESON
MICHAEL R. GRAHAM
ROGER A. HEPPELMANN
DANIELLE M. JOHNSTON
GREGORY C. MAYER
WILLIAM K. MERKEL, Ph.D.
STEPHEN M. MILLER
LI-HSIEN RIN-LAUREN, M.D.
DOUGLAS H. SEGEL
YOUNG J. SUH, Ph.D.

BY COUNSEL
JOHN H. COULT

REGISTERED PATENT AGENTS
GRETA E. NOLAND
JOSEPH A. WILLIAMS, JR., Ph.D.

July 17, 1996

FACSIMILE
TRANSMISSION SHEET

TO: Tim Heightbrink
FAX NO.: (703) 308-6916

CLIENT NO.: 28113

MATTER NO.: 33072

FROM: David A. Gass

COUNTRY CODE: US

FAX NO.: (312) 474-0448

PAGES (INCLUDING THIS PAGE): 4

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JUL 17 1996

PATENT OFFICE

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The material of this transmission contains confidential information intended only for the addressee. If you are not the addressee, any disclosure or use of this information by you is strictly prohibited. If you have received this facsimile in error, please notify us by telephone immediately.





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 7

David A. Gass
Marshall, O'Toole, Gerstein,
Murray & Borun
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402

COPY MAILED

JUL 22 1996

**OFFICE OF PETITIONS
AND DOCKETING**

In re Application of
Alitalo et al.
Application No. 08/585,895
Filed: January 12, 1996
Attorney Docket No. 28113/33072

DECISION GRANTING PETITION

This is a decision on the petition filed May 24, 1996 made special by the petition filed by "Express Mail" June 25, 1996 (copy of the July 25, 1996 petition sent July 17, 1996, by facsimile transmission in response to a telephone communication by Tim Heitbrink, of the Office of Petitions), requesting that the above-identified application be accorded a filing date of January 12, 1996.

The application, which is a continuation-in-part application under 37 CFR 1.53, was deposited in Express Mail service on January 12, 1996, which was a Friday. The Express Mail label number was placed on the papers. However, Federal and District of Columbia government offices, including the Patent and Trademark Office (Office), were officially closed for the entire day on January 12, 1996, as a result of adverse weather conditions. Under such conditions, the Office considers that day as a "federal holiday within the District of Columbia" under 35 U.S.C. 21. See 1183 OG 60 and notice entitled "Filing Of Papers During Unscheduled Closings Of The Patent and Trademark Office", originally published at 1097 OG 53, reprinted at 1158 OG 8 (copies enclosed).

In accordance with Office procedure, the present application was accorded a filing date of Tuesday, January 16, 1996, the next business day following the date of deposit in Express Mail service. The fact that no papers are received or stamped on Saturdays, Sundays or Federal holidays within the District of Columbia and the handling of Express Mail in such cases is clearly set out in 37 CFR 1.6(a) and 1.10(a).

Of course, "in an extraordinary situation, where justice requires" the Commissioner may waive or suspend the rules and accord this application a January 12, 1996 filing date pursuant

Application No. 08/585,895

Page 2

to 37 CFR 1.183. In this case, petitioners request waiver of the rules under 37 CFR 1.183 based on the need to protect applicants' patent rights in foreign countries.

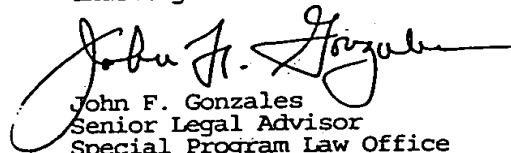
Under the circumstances of this particular case, it is deemed appropriate to waive the rules pursuant to 37 CFR 1.183 in order to accord the application a filing date of January 12, 1996.

The petition under 37 CFR 1.183 is granted.

Receipt is acknowledged of the combined declaration and power of attorney filed April 1, 1996.

The application is being returned to Application Processing Division for further processing with a filing date of January 12, 1996.

Any inquiries related to this decision should be directed to Tim Heitbrink at (703) 308-6713, or if not available, to the undersigned at (703) 305-9282.



John F. Gonzales
Senior Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

twh

Enclosure: 1183 OG 60
1158 OG 8



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo et al.

Serial No: 08/585,895

Filed: January 12, 1996

Title: RECEPTOR LIGAND

Group Art Unit: Not yet assigned

Examiner: Not yet assigned



I hereby certify that this paper is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C., 20231 on this date:

Date: August 12, 1996

David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary Amendment prior to examination of the above-identified application on the merits by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

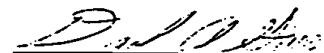
At page 1, line 3, after "August 1, 1995.", please insert -- This application is also a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994.--

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: August 17, 1977



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|--------------------------------------|---|---------------------------------------|
| In the Application of: Alitalo, Kari |) | I hereby certify that this paper and |
| and Joukov, Vladimir |) | the documents referred to as enclosed |
| Serial No.: 08/585,895 |) | herewith are being deposited with the |
| Filed: January 12, 1996 |) | United States Postal Service as First |
| For: "Receptor Ligand" |) | Class Mail, postage prepaid, in an |
| Group Art Unit: 1806 |) | envelope addressed to: Assistant |
| Examiner: To be determined |) | Commissioner for Patents, |
| |) | Washington, DC 20231, on this date: |
| |) | October 14, 1996 |
| |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| |) | Reg. No.: 38,153 |
| |) | Attorney for Applicants |

**INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

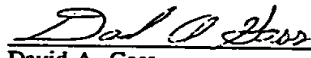
Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

October 14, 1996

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| and Joukov, Vladimir |) | the documents referred to as enclosed |
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| For: "Receptor Ligand" |) | Commissioner for Patents, |
| |) | Washington, DC 20231, on this date: |
| Group Art Unit: 1806 |) | October 14, 1996 |
| |) |  |
| Examiner: To be determined |) | David A. Gass |
| |) | Reg. No.: 38,153 |
| |) | Attorney for Applicants |

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of each listed document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.


This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A copy of this paper is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

October 14, 1996

By:


David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|--------------------|-------------|-----------------------|---------------------|
| 02/585,895 | 01/12/96 | ACUT-LE | K 28113/30072 |

19M2/1125
MARSHALL O'TOOLE GERSTEIN MURRAY & BORUM
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

| EXAMINER | |
|-----------|--------------|
| LAWSON, B | |
| ART UNIT | PAPER NUMBER |
| 1801 | 6 |

DATE MAILED: 11/25/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☐ Responsive to communication(s) filed on _____
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 0 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-16 is/are pending in the application.
- Of the above, claim(s) _____ is/are withdrawn from consideration.
- ☐ Claim(s) _____ is/are allowed.
- ☐ Claim(s) _____ is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☒ Claims 1-16 are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been
- ☐ received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Serial Number: 08/585895
Art Unit: 1801

2

DETAILED ACTION

Election/Restriction

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:
 - I. Claims 1, 2, 8-10, 12, and 16, drawn to a ligand for the Flt4 receptor and compositions comprising the same, classified in class 530, subclass 399.
 - II. Claims 3-7, 11, and 13, drawn to nucleic acids encoding a ligand for the Flt4 receptor and vectors and host cells comprising the same, classified in class 536, subclass 23.51.
 - III. Claims 14-15, drawn to an antibody specifically reactive to a ligand for the Flt4 receptor, classified in class 530, subclass 387.1.
2. The inventions are distinct, each from the other because of the following reasons:
3. The protein of Group I is a patentably distinct chemical species from the nucleic acids of Group II, although related as the nucleic acids encode the protein. The protein can be made without recourse to the nucleic acids by the materially distinct process of biochemical purification from tissue or serum, and the nucleic acids have separate utility as probes for screening expression libraries.
4. The protein of Group I is a patentably distinct chemical species from the antibody of Group III, although related as the antibody can bind the protein. The antibody can cross-react with other proteins, and other antibodies can cross-react with the protein. The protein can be

made without recourse to the antibody by the materially distinct process of biochemical purification from tissue or serum, and the protein has separate utility as a therapeutic agent.

5. The antibodies of Group III are patentably distinct from the nucleic acids of Group II, although related as the antibodies may be raised against proteins encoded by the nucleic acids. The inventions have distinct chemical compositions and distinct functions. The nucleic acids are not required to make the antibodies, which may be raised against proteins made without recombinant expression. The nucleic acids have separate utility as probes, for example.

6. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, restriction for examination purposes as indicated is proper.

7. A telephone call was made to David Gass on 14 November 1996 to request an oral election to the above restriction requirement, but did not result in an election being made.

Applicant is advised that the response to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

8. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(h).

Serial Number: 08/585895
Art Unit: 1801

4

Conclusion

9. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Vasu Jagannathan, can be reached at (703) 306-2777. The FAX number for Art Unit 1801 is (703) 305-7401.

An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.

Art Unit 1801

Vasu Jagannathan
SPE
AU 1801

1806

038° T/8

PATENT #
28113/33072 G.
12-1
prel

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|----------------------------------|---|---------------------------------------|
| Applicants: Alitalo et al. |) | I hereby certify that this paper is |
| Serial No: 08/585,895 |) | being deposited with the United |
| Filed: January 12, 1996 |) | States Postal Service with sufficient |
| Title: RECEPTOR LIGAND |) | postage as first class mail in an |
| Group Art Unit: Not yet assigned |) | envelope addressed to: Assistant |
| Examiner: Not yet assigned |) | Commissioner for Patents, |
| |) | Washington, D.C., 20231 on this |
| |) | date: |
| |) | Date: <u>August 12, 1996</u> |
| |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| |) | Registration No. 38,153 |
| |) | Attorney for Applicants |

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary
Amendment prior to examination of the above-identified application on the merits
by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

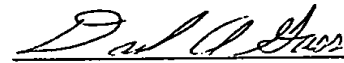
At page 1, line 3, after "August 1, 1995.", please insert This
A₁ application is also a continuation-in-part of U.S. Patent Application Serial No.
08/340,011, filed November 14, 1994.

REMARKS

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

Respectfully submitted,

Dated: August 12, 1996



David A. Gass
Registration No. 38,153

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 S. Wacker Drive
Chicago, Illinois 60606
Telephone: (312) 474-6300



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|----------------------------------|
| Applicant(s): |) | Title: Receptor Ligand |
| |) | |
| Alitalo et al. |) | |
| |) | |
| Serial No: 08/585,895 |) | Group Art Unit: Not yet assigned |
| |) | |
| Filed: January 12, 1996 |) | Examiner: Not yet assigned |
| |) | |

TRANSMITTAL LETTER

*Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith are a Preliminary Amendment and an executed Inventors' Declaration for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on August 12, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
☐ Small entity status has been established and is still effective.
☒ Has not been established.

2. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: August 12, 1996

By: David A. Gass

David A. Gass
Reg. No: 38,153



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|----------------------------------|
| Applicant(s): |) | Title: Receptor Ligand |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: Not yet assigned |
| Filed: January 12, 1996 |) | Examiner: Not yet assigned |

TRANSMITTAL LETTER

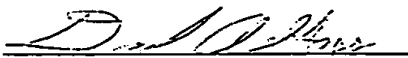
**Assistant Commissioner for Patents
Washington, D.C. 20231**

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MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: August 13, 1996

By: David A. Gass

David A. Gass
Reg. No: 38,153



Atty. Docket No: 28113/33072

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed
☐ ☒

| | | |
|-----------------------------|-----------|------------------------|
| 950674 | Finland | 13 February 1995 |
| (Application Serial Number) | (Country) | (Day/Month/Year Filed) |

Yes No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| | |
|-----------------------------|------------------------|
| (Application Serial Number) | (Day/Month/Year Filed) |
|-----------------------------|------------------------|

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

| | | |
|-----------------------------|------------------------|---|
| 08/340,011 | 14 November 1994 | Pending |
| (Application Serial Number) | (Day/Month/Year Filed) | (Status-Patented, Pending or Abandoned) |
| 08/510,133 | 01 August 1995 | Pending |
| (Application Serial Number) | (Day/Month/Year Filed) | (Status-Patented, Pending or Abandoned) |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)
Donald J. Brott (19,490)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)

Trevor B. Joike (25,542)
Timothy J. Vazquez (26,348)
Carl E. Moore, Jr. (26,447)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)

Richard A. Schurr (30,890)
Anthony Nimmo (30,920)
Christine A. Dudzik (31,245)
Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Donald J. Pochopien (32,167)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. Le Barge (32,254)
Jeffrey W. Smith (33,455)
Douglas C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)
David A. Gass (38,153)

Send correspondence to: David A. Gass

| FIRM NAME | PHONE NO. | STREET | CITY & STATE | ZIP CODE |
|--|--------------|--|-------------------|------------|
| Marshall, O'Toole, Gerstein, Murray & Borun | 312-474-6300 | 6300 Sears Tower 233 South Wacker Drive | Chicago, Illinois | 60606-6402 |

| | |
|-------------------------------------|------------------------------|
| Full Name of First or Sole Inventor | Citizenship |
| Kari Alitalo | Finland |
| Residence Address - Street | Post Office Address - Street |
| Nyyrikintie 4A | Same |
| City (Zip) | City (Zip) |
| 02100 Espoo | Same |
| State or Country | State or Country |
| FINLAND | Same |
| Date | Signature |
| Aug 6 1996 | [Signature] |

☒ See second page for additional inventor

See reverse for relevant rules & statutes

| | |
|--|---|
| Second Joint Investor, if Any Vladimir Joukov | Citizenship Russia |
| Residence Address - Street Topeliuksenkatu 32G8 | Post Office Address - Street Same |
| City (Zip) 00290 Helsinki | City (Zip) Same |
| State or Country FINLAND <i>Fin</i> | State or Country Same |
| Date <input checked="" type="checkbox"/> Aug. 6, 1996 | Signature <input checked="" type="checkbox"/> <i>V. Joukov</i> |

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PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|--|
| In the Application of: |) | I hereby certify that this paper and the |
| |) | documents referred to as enclosed |
| Alitalo et al. |) | herewith are being deposited with the |
| |) | United States Postal Service as First |
| Serial No.: 08/585,895 |) | Class Mail, postage prepaid, in an |
| |) | envelope addressed to: Assistant |
| Filed: January 12, 1996 |) | Commissioner for Patents, Washington, |
| |) | DC 20231, on this date: |
| For: "Receptor Ligand" |) | |
| |) | Date: <u>December 19, 1996</u> |
| Group Art Unit: 1801 |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| Examiner: Lathrop, B. |) | Reg. No.: 38,153 |
| |) | Attorney for Applicant |

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVED
JAN 10 1997
GROUP 1800

Sir:

The Applicants request that the documents listed on the attached Form PTO-1449 be made of official record in the above-identified application. A copy of the listed documents are enclosed herewith.

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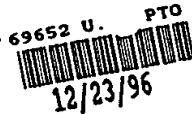
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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Alitalo et al. |) | documents referred to as enclosed |
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| For: "Receptor Ligand" |) | Class Mail, postage prepaid, in an |
| Group Art Unit: 1801 |) | envelope addressed to: Assistant |
| Examiner: Lathrop, B. |) | Commissioner for Patents, Washington, |
| |) | DC 20231, on this date: |
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| |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| |) | Reg. No.: 38,153 |
| |) | Attorney for Applicant |

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

RECEIVED
JAN 10 1997
GROUP 1800

Assistant Commissioner for Patents
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MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: December 19, 1996

By: David A. Gass

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



#13
I.D.S. 4/29/97
PATENT
28113/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
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| In the Application of: |) | I hereby certify that this paper and the |
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| Filed: January 12, 1996 |) | Commissioner for Patents, Washington, |
| |) | DC 20231, on this date: |
| For: "Receptor Ligand" |) | |
| |) | Date: 21 January 1997 |
| Group Art Unit: 1801 |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| Examiner: Lathrop, B. |) | Reg. No.: 38,153 |
| |) | Attorney for Applicant |

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

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MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: 21 Jan. 1997

By: David A. Gass

David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
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PATENT
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| Examiner: Lathrop, B. |) | Commissioner for Patents, Washington, |
| |) | DC 20231, on this date: |
| |) | Date: <u>21 January 1997</u> |
| |) | <u>David A. Gass</u> |
| |) | David A. Gass |
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| |) | Attorney for Applicant |

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Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300



THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996


For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is
being deposited with the United
States Postal Service as first class
mail, postage prepaid, in an
envelope addressed to: Assistant
Commissioner for Patents,
Washington, D.C. 20231, on this
date:

Dated: 24 Jan. 1997


David A. Gass

AMENDMENT AND ELECTION IN RESPONSE TO RESTRICTION REQUIREMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In an official communication dated November 25, 1996, the U.S. Patent and Trademark Office issued a restriction requirement in the above-identified patent application, and set a 30 day period for response. This amendment and election in response to the restriction requirement has been timely filed with a petition for one month extension of time and petition fee, extending the time for response until January 25, 1997.

RECEIVED
JAN 27 1997

AMENDMENTS

In the claims:

Please cancel claims 2, 8-10, 12, and 14-16 without prejudice; amend claims 1, 3, 5, 11, and 13; and add new claims 17-25 to the application as shown below.

B1 sub
C4

1. (Amended) A purified and isolated polynucleotide encoding a polypeptide which specifically binds to the Flt4 receptor tyrosine kinase.

B2 sub
C5

3. (Amended) A purified and isolated nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33. [the peptide according to claim 2.]

B3 sub
C5

5. (Amended) A vector comprising the nucleic acid according to claim 3 [4].

B4 sub
C7

11. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises approximately amino acids 1 to 120 of SEQ ID NO: 33. [encoding the fragment of claim 10.]

B5

13. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises approximately amino acids 1 to 180 of SEQ ID NO: 33. [encoding the fragment of claim 12.]

B6

-17. A host cell transformed or transfected with a vector according to claim 5.

sub
C8

18. A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33, said portion encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase.

Sub
B6
sub
C9

19. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of FIt4-receptor tyrosine kinase.

20. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 kd as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.--

REMARKS

This is the Applicants' second amendment to this application. A preliminary amendment, to add a priority claim to the application, was mailed on August 12, 1996.

The Applicants do not intend by the foregoing amendments or any other amendments to abandon the subject matter of any claim as originally filed or later amended, and reserve the right to claim such subject matter in other

applications, such as continuations, continuations-in-part, and divisional applications.

I. The Applicants Elect Claims 3-7, 11, and 13 (Group II) without traverse.

In response to the restriction requirement, the Applicants hereby elect Group II (Claims 3-7, 11, and 13), drawn to nucleic acids, vectors, and host cells.

II. Explanation of amendments.

Claim 1 has been amended to recite a nucleic acid, rather than a polypeptide, thereby bringing claim 1 within the scope of the elected invention of Group II.

Claim 3, which formerly depended from non-elected claim 2, has been amended to be an independent claim. Claims 11 and 13 have been amended similarly.

New claims 18 and 19 find support in Example 4 (pp. 19-21), for example.

New claim 20 finds support in Example 5 (p. 22, lines 33-34), for example.

New claim 21 finds support in Example 5 at p. 23, lines 9-10, for example.

New claim 22 finds support at p. 23, lines 9-10, and p. 30, lines 14-17, for example.

New claim 23 finds support in Example 13 (p. 31, lines 33-34), for example.

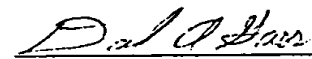
All of the pending claims (as amended herein) are properly classified with the Group II claims elected by the Applicants in response to the restriction requirement. None of the new claims introduce new matter.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan. 1997

By:


David A. Gass
Reg. No: 38,153



H. 14
E.H. O'Flaherty
4/29/97
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1801 |
| Filed: January 12, 1996 |) | Examiner: Lathrop, B. |

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

*Assistant Commissioner for Patents
Washington, D.C. 20231*


Sir:

Transmitted herewith is an amendment for the above application.

C 010 1 00585895 00210 970100 070004 115 119.00

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on January 24, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | | FEE FOR LARGE ENTITY | | FEE FOR SMALL ENTITY |
|-----------------------|---|----------------------|--|----------------------|
| One Month | x | \$110.00 | | \$55.00 |
| Two Months | | \$390.00 | | \$195.00 |
| Three Months | | \$930.00 | | \$465.00 |
| Four Months | | \$1,470.00 | | \$735.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$110.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$_____

Extension Fee Due With This Request \$110.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

| | | | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
|---|----------------------------------|---------------------------------|----|---------------|--------------|----------------|---------------------------|----------------|
| | Claims Remaining After Amendment | Highest No. Previously Paid For | | Present Extra | Rate | Additional Fee | Rate | Additional Fee |
| TOTAL | 17 | MINUS | 20 | = 0 | X11 = | \$ | X22 = | \$ |
| INDEP. | 3 | MINUS | 3 | = 0 | X40 = | \$ | X80 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | | | +130 = | \$ | +260 = | \$ |
| TOTAL ADDITIONAL FEE | | | | | | \$ | OR | \$100.00 |

4. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$110.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan. 1997

By: David A. Gass
David A. Gass
Reg. No: 38,153



1800

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1801 |
| Filed: January 12, 1996 |) | Examiner: Lathrop, B. |

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PETITION FOR EXTENSION OF TIME**


*Assistant Commissioner for Patents
Washington, D.C. 20231*

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David A. Gass

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Extension Fee: \$110.00

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Extension Fee Due With This Request \$110.00

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| | | | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
|---|----------------------------------|---------------------------------|----|---------------|--------------|----------------|---------------------------|----------------|
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| INDEP. | 3 | MINUS | 3 | = 0 | X40 = | \$ | X80 = | \$ |
| <input type="checkbox"/> First Presentation of Multiple Dependent Claim | | | | | + 130 = | \$ | + 260 = | \$ |
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Respectfully submitted,

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MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: 24 Jan, 1997

By: David A. Gass
David A. Gass
Reg. No: 38,153



Gp. 1801
PATENT APPLICATION
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

The Application of: Alitalo, Kari
and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

#11
"EXPRESS MAIL"
Mailing label No. EMO99898621US
Date of Deposit: February 11, 1997
I hereby certify that this paper and the
documents referred to as enclosed herewith are
being deposited with the United States Postal
Service "EXPRESS MAIL POST OFFICE TO
ADDRESSEE" service under 37 CFR §1.10 on
the date indicated above and is addressed to:
Assistant Commissioner for Patents,
Washington, D.C. 20231
Mark Bonadonna
Mark Bonadonna

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each document is enclosed herewith.

This Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

This Information Disclosure Statement is submitted before receipt of a first Office action on the merits, and consequently should be considered by the Patent Office without payment of a fee. See 37 C.F.R. §1.97(b). However, please charge any necessary fees due in connection with this Information Disclosure Statement to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: *David A. Gass*
David A. Gass
Registration No.: 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Feb 11, 1997

PATENT APPLICATION
23967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitale, K. and Joukov, Vladimir

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.



"EXPRESS MAIL"

Mailing label No. EMO99898621US

Date of Deposit: February 11, 1997

I hereby certify that this paper and the documents referred to as enclosed herewith are being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 CFR §1.10 on the date indicated above and is addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

Mark Bonadonna
Mark Bonadonna

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

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FEB 11 1997
GROUP 12

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each document is enclosed herewith.

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: *David A. Gass*
David A. Gass
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6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Feb 11, 1997

| | | | | |
|--|---|--|---------------------------------|--------------------------|
| Form PTO-1449 (Modified) |  | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | | Applicant Alitalo and Joukov | |
| | | | Filing Date 01/12/96 | Group 1801 |

U.S. PATENT DOCUMENTS

| *Examiner Initials | Document Number | Issue Date | Name | Class | Subclass | Filing Date If Appropriate |
|-----------------------|--------------------|---------------|------|-------|----------|----------------------------------|
| | | | | | | |

FOREIGN PATENT DOCUMENTS

| *Examiner Initials | Document Number | Publication Date | Country | Class | Subclass | Translation | |
|-----------------------|--------------------|---------------------|---------|-------|----------|-------------|----|
| | | | | | | Yes | No |
| | | | | | | | |

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

| | | |
|-----------|------|---|
| <i>Al</i> | C111 | Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22). |
| | C112 | Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C and Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation (GAAC)/ Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT). |
| | C113 | Kukk <i>et al.</i> , "VEGF-C Receptor Binding and Pattern of Expression with VEGFR-2 Suggests a Role in Lymphatic Vascular Development," <i>Development</i> , 122:3829-3837 (1996). |
| | C114 | Paavonen <i>et al.</i> , "Chromosomal Localization and Regulation of Human Vascular Endothelial Growth Factors B and C (VEGF-B and VEGF-C)," <i>IX International Vascular Biology Meeting</i> , Seattle, Washington, September 4-8, 1996, p. 76 (ABSTRACT 299). |

| | |
|---|----------------------------------|
| EXAMINER <i>Erica Lathrop</i> | DATE CONSIDERED <i>5/2/97</i> |
| *EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. | |

SHEET 1

| | | | | |
|--|--|--|---------------------------------|--------------------------|
| Form PTO-1449 (Modified) | | U.S. Department of Commerce Patent and Trademark Office | Any. Docket No. 28967/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | | Applicant Alitalo and Joukov | |
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|-----------------------|--------------------|---------------------|---------|-------|----------|-------------|----|
| | | | | | | Yes | No |
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| | | |
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| | C111 | Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD), Development, Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22). |
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EXAMINER

DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



GP 1801
PATENT APPLICATION #
28967/33072

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996

For: "Receptor Ligand"

Group Art Unit: 1801

Examiner: Lathrop, B.

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail, postage prepaid, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this date:

Dated: March 21, 1997

David A. Gass
David A. Gass
Registration No. 38,153
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

RECEIVE
APR 10 1997
GROUP 1801

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed document, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of the document is enclosed herewith.

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Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

Date: March 21, 1997

By: David A. Gass
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PATENT APPLICATION
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Attorney for Applicant

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Assistant Commissioner for Patents
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MURRAY & BORUN

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Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAtty. Docket No.
28967/33072Serial No.
08/585,895

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

Applicant
Alitalo and JoukovFiling Date
01/12/96Group
1801

U.S. PATENT DOCUMENTS

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|-----------------------|--------------------|---------------|------|-------|----------|----------------------------------|
| | | | | | | |
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FOREIGN PATENT DOCUMENTS

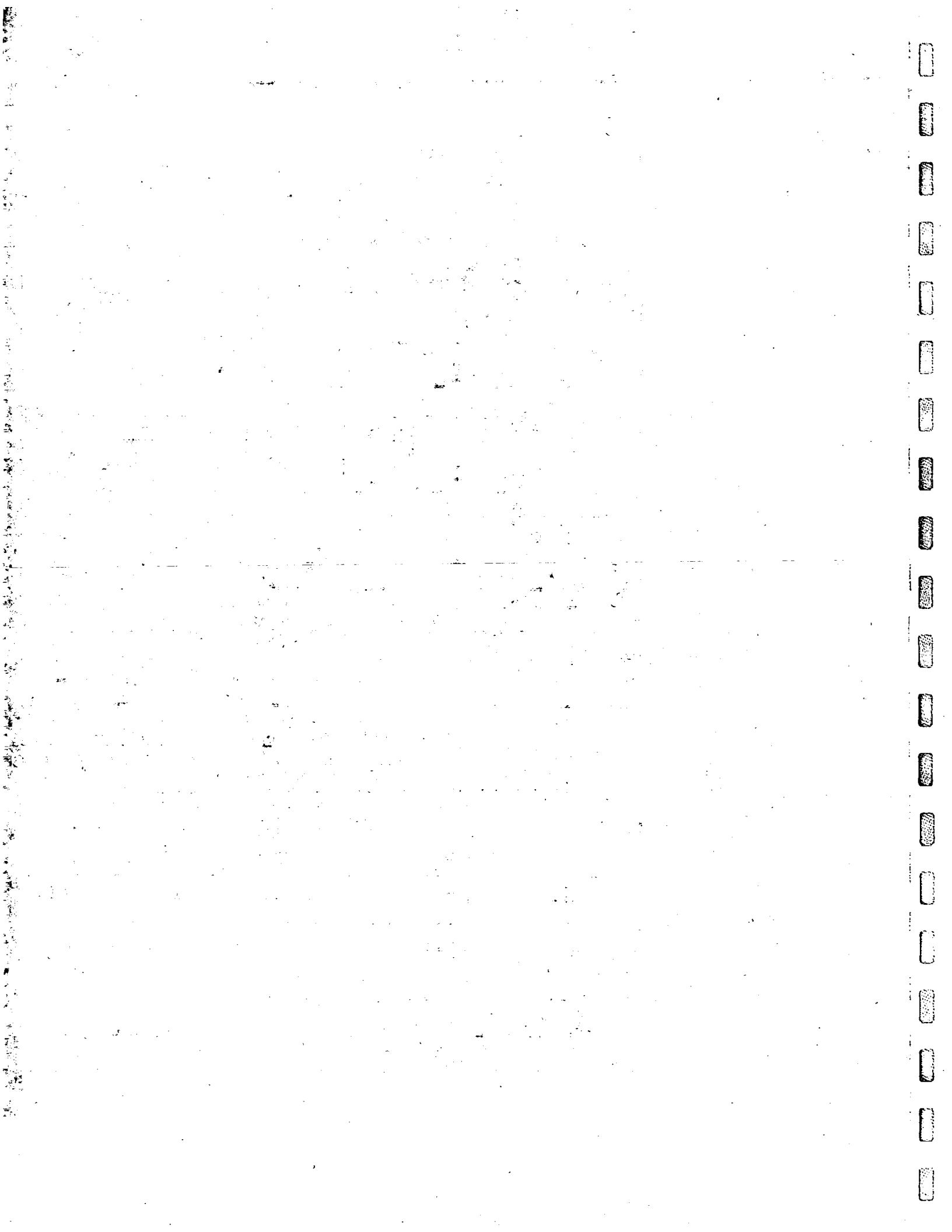
| *Examiner Initials | Document Number | Publication Date | Country | Class | Subclass | Translation | |
|-----------------------|--------------------|---------------------|---------|-------|----------|-------------|----|
| | | | | | | Yes | No |
| | | | | | | | |

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

| | | |
|------------|------|--|
| <i>Enl</i> | C115 | Pajusola, "Cloning and Characterization of a New Endothelial Receptor Tyrosine Kinase Flt4 and Two Novel VEGF-Like Growth Factors VEGF-B and VEGF-C," Academic Dissertation, Molecular/Cancer Biology Laboratory and Department of Pathology, Haartman Institute and Department of Biosciences, Division of Genetics, University of Helsinki, (January 26, 1996) |
| | | |
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EXAMINER *Brian Lathrop*DATE CONSIDERED *5/5/97*

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.





IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT
28967/33072

Gp 1801
#10
5/17

In the Application of:
Alitalo et al.
Serial No.: 08/585,895
Filed: January 12, 1996
For: "Receptor Ligand"
Group Art Unit: 1801
Examiner: Lathrop, B.

I hereby certify that this paper is being
deposited with the United States Postal
Service as first class mail, postage prepaid,
in an envelope addressed to: Assistant
Commissioner for Patents, Washington, D.C.
20231, on this date:
Dated: Apr 11 1997
David A. Gass
Registration No. 38,153
Attorney for Applicant

INFORMATION DISCLOSURE STATEMENT
PURSUANT TO 37 C.F.R. §§1.56, 1.97, AND 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each itemized document is enclosed herewith. Both of the documents were identified in an International Search Report (ISR, copy enclosed herewith) in a related PCT patent application. Documents identified in the ISR that are not itemized on the attached Form PTO-1449 have already been made of record by the Patent Office or the applicants.

This Information Disclosure Statement is not intended to be an admission that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 37 U.S.C. §102 or §103.

Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached form PTO-1449 was cited in a

communication (an ISR) from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: David A. Gass
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: April 16, 1997



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


For: "Receptor Ligand"

Group Art Unit: 1801

Examjner: Lathrop, B.

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage prepaid,
) in an envelope addressed to: Assistant
) Commissioner for Patents, Washington, D.C.
) 20231, on this date:

) Dated: April 16, 1997

) 
) David A. Gass
) Registration No. 38,153
) Attorney for Applicant

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Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached form PTO-1449 was cited in a



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MAY 6 1997

communication (an ~~EP~~ from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN

By: David A. Gass
David A. Gass
Registration No. 38,153
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

Date: April 16, 1997

Form PTO-1449 (Modified)

U.S. Department of Commerce
Patent and Trademark OfficeAtty. Docket No.
28967/33072Serial No.
08/585,895Applicant
Alitalo et al.Filing Date
01/12/96Group
1801

INFORMATION DISCLOSURE STATEMENT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

| *Examiner Initials | Document Number | Issue Date | Name | Class | Subclass | Filing D If Appropri |
|-----------------------|--------------------|---------------|------|-------|----------|----------------------------|
| | | | | | | |
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FOREIGN PATENT DOCUMENTS

| *Examiner Initials | Document Number | Publication Date | Country | Class | Subclass | Translation | |
|-----------------------|--------------------|---------------------|----------|-------|----------|-------------|----|
| | | | | | | Yes | No |
| B7 | WO 95/33772 | 12/14/95 | PCT in D | - | - | | |
| | | | | | | | |
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OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

| | | |
|----|------|--|
| B7 | C116 | Hillier et al., "The WashU-Merck EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995. |
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| | | |
| | | |

EXAMINER

Brian Lashrop

DATE CONSIDERED

5/11/97

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.





UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| | | | |
|--------------------|-------------|-----------------------|---------------------|
| APPLICATION NUMBER | FILING DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NO. |
|--------------------|-------------|-----------------------|---------------------|

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| EXAMINER |
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| ART UNIT | PAPER NUMBER |
|----------|--------------|

17

DATE MAILED:

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

☒ Responsive to communication(s) filed on 1/27/97 (Election/Amendment)

☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) 1, 3-7, 11, 13, 17-25 is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☒ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.

☒ The specification is objected to by the Examiner.

☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) _____

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of Reference Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 8, 9, 11-13, 16

☐ Interview Summary, PTO-413

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☒ Notice to Comply - SEE OFFICE ACTION ON THE FOLLOWING PAGES -

Application No.: 08/585895; attachment to Paper No.
**NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING
NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES**

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

- ☒ 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.
- ☐ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).
- ☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).
- ☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."
- ☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).
- ☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).
- ☒ 7. Other: Figure 10 contains sequences requiring a SEQ ID NO, and Figure 9B requires identification of protein and polynucleotide sequences by SEQ ID NO: 32 and 33. SEQ ID NOS may be added to the Brief Description of the Drawings or the Figures.

Applicant Must Provide:

- ☒ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".
- ☒ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.
- ☒ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
For CRF Submission Help, call (703) 308-4212
For Patent software help, call (703) 308-6856

PLEASE RETURN A COPY OF THIS NOTICE WITH YOUR RESPONSE

Serial Number: 08/585895
Art Unit: 1801

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DETAILED ACTION

Election/Restriction

1. Applicant's election without traverse of Group II, claims 3-7, 11, and 13, and amendment
5 of claim 1 to read on the elected invention in Paper No. 15 is acknowledged.

Oath/Declaration

2. The oath or declaration is defective. A new oath or declaration in compliance with 37
CFR 1.67(a) identifying this application by application number and filing date is required. See
10 MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed alterations have been made to the oath or declaration. See 37 CFR 1.52(c)
and 1.57).

15 It does not state that the person making the oath or declaration in a continuation-in-part
application filed under the conditions specified in 35 U.S.C. 120 which discloses and
claims subject matter in addition to that disclosed in the prior copending application,
acknowledges the duty to disclose to the Office all information known to the person to be
20 material to patentability as defined in 37 CFR 1.56 which occurred between the filing date
of the prior application and the national or PCT international filing date of the
continuation-in-part application.

Serial Number: 08/585895
Art Unit: 1801

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(e) the deposit will be replaced should it become necessary due to inviability, contamination, or loss of capability to function described in the manner in the specification.

5 In either case, the identifying information set forth in 37 C.F.R. 1.809(d) should be added to the specification if it is not already present. See 37 C.F.R. 1.803-1.809 for additional explanation of these requirements.

8. Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for
10 polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides all polypeptides that bind the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

15 The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4
20 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor without some information on the structure of the protein, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention. There is no guidance provided by the state of the art to select ligands to make the invention; despite intense

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Art Unit: 1801

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research in this area, Borg et al. (reference C7) teach that no known ligands for the Flt4 receptor were known at the time of the invention. The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

10

9. Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

15

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only

20

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Art Unit: 1801

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guidance offered by the specification to chose fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*). Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make

081

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Art Unit: 1801

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the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

10. Claims 3-5, 11, 13, and 17-25 are rejected under 35 U.S.C. 112, second paragraph, as
5 being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome
10 Sciences, Inc, disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as "residue 1" would have meant that this residue was the amino terminus of the mature polypeptide;
15 however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. *MPEP* 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n.6., 181 USPQ 31, 34 n.6 (CCPA 1974).

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11. Claims 11 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

Allowable Subject Matter

12. Claims 3-5, 11, 13, and 17 would be allowable over the prior art of record if rewritten or amended to overcome the rejection(s) under 35 U.S.C. 112 set forth in this Office action.

13. The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc., reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs

Serial Number: 08/585895
Art Unit: 1801

-10-

encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-STS Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

5

Conclusion

14. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

- Eriksson et al. disclose VEGF-B and DNA encoding this protein, which appears to be structurally distinct from the instantly claimed DNAs. It does not appear that the DNAs disclosed by Eriksson et al. encode proteins that would reasonably have the inherent property of meeting the claim limitation of binding the Flt4 receptor.

10

15. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

15

The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Stephen Walsh, can be reached at (703) 308-2957. The FAX number for Art Unit 1801 is (703) 305-7401.

20

An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.


Brian K. Lathrop, Ph.D.
5/25/97


DAVID L. FITZGERALD
PRIMARY EXAMINER
GROUP 1800

\$ 58589

NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 1/12/96A. ☒ not objected to by the Draftsperson under 37 CFR 1.84 or 1.152.

B. ☒ objected to by the Draftsperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:

Black ink. Color.

☐ Not black solid lines. Fig(s) _____☐ Color drawings are not acceptable until petition is granted.

Fig(s) _____

2. PHOTOGRAPHS. 37 CFR 1.84(b)

☒ Photographs are not acceptable until petition is granted.

Fig(s) _____

☐ Photographs not properly mounted (must use bristol board or photographic double-weight paper). Fig(s) _____☐ Poor quality (half-tone). Fig(s) _____

3. GRAPHIC FORMS. 37 CFR 1.84(d)

☐ Chemical or mathematical formula not labeled as separate figure.

Fig(s) _____

☐ Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis.

Fig(s) _____

☐ Individuals waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) _____

4. TYPE OF PAPER. 37 CFR 1.84(c)

☐ Paper not flexible, strong, white, smooth, nonshiny, and durable.

Sheet(s) _____

☐ Erasures, alterations, overwritings, interlineations, cracks, creases, and folds copy machine marks not accepted. Fig(s) _____☐ Mylar, velum paper is not acceptable (too thin). Fig(s) _____

5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:

☐ 21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)☐ 21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)☐ 21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)☐ 21.0 cm. by 29.7 cm. (DIN size A4)☐ All drawing sheets not the same size. Sheet(s) _____☐ Drawing sheet not an acceptable size. Sheet(s) _____

6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

Paper size

21.6 cm. X 35.6 cm. 21.6 cm. X 33.1 cm. 21.6 cm. X 27.9 cm. 21.0 cm. X 29.7 cm.

(8 1/2 X 14 inches) (8 1/2 X 13 inches) (8 1/2 X 11 inches) (DIN Size A4)

T 5.1 cm. (2") 2.5 cm. (1") 2.5 cm. (1") 2.5 cm.

L .64 cm. (1/4") .64 cm. (1/4") .64 cm. (1/4") 1.5 cm.

R .64 cm. (1/4") .64 cm. (1/4") .64 cm. (1/4") 1.5 cm.

B .64 cm. (1/4") .64 cm. (1/4") .64 cm. (1/4") 1.0 cm.

Margins do not conform to chart above.

Sheet(s) _____

Top (T) _____ Left (L) _____ Right (R) _____ Bottom (B) _____

7. VIEWS. 37 CFR 1.84(h)

REMINDER: Specification may require revision to correspond to drawing changes.

☐ All views not grouped together. Fig(s) _____☐ Views connected by projection lines or lead lines.

Fig(s) _____

☐ Partial views. 37 CFR 1.84(h) 2☒ View and enlarged view not shown separately or properly.

Fig(s) _____

☐ Sectional views. 37 CFR 1.84 (h) 3☐ Hatching not indicated for sectional portions of an object.

Fig(s) _____

☐ Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) _____

8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

☐ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) _____

9. SCALE. 37 CFR 1.84(k)

☐ Scale not large enough to show mechanism with crowding when drawing is reduced in size to two-thirds in reproduction.

Fig(s) _____

☐ Indication such as "actual size" or scale 1/2" not permitted.

Fig(s) _____

10. CHARACTER OF LINES, NUMBERS, & LETTERS. 37 CFR 1.84(j)

☐ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings).

Fig(s) _____

11. SHADING. 37 CFR 1.84(m)

☐ Solid black shading areas not permitted.

Fig(s) _____

☐ Shade lines, pale, rough and blurred. Fig(s) _____

12. NUMBERS, LETTERS, & REFERENCE CHARACTERS. 37 CFR 1.84(p)

☐ Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) _____☐ Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) _____☐ English alphabet not used. 37 CFR 1.84(p)(2)

Fig(s) _____

☐ Numbers, letters, and reference characters do not measure at least .32 cm. (1/8 inch) in height. 37 CFR(p)(3)

Fig(s) _____

13. LEAD LINES. 37 CFR 1.84(q)

☐ Lead lines cross each other. Fig(s) _____☐ Lead lines missing. Fig(s) _____

14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(i)

☐ Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) _____

15. NUMBER OF VIEWS. 37 CFR 1.84(u)

☐ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) _____☐ View numbers not preceded by the abbreviation Fig.

Fig(s) _____

16. CORRECTIONS. 37 CFR 1.84(w)

☐ Corrections not made from prior PTO-948.

Fig(s) _____

17. DESIGN DRAWING. 37 CFR 1.152

☐ Surface shading shown not appropriate. Fig(s) _____☐ Solid black shading not used for color contrast.

Fig(s) _____

COMMENTS:

- Fig legend placed incorrectly (Fig 9A, 15A, 16A)

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| EXAMINER Brian Lathrop | DATE CONSIDERED 5/8/97 |
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| Form PTO-1449 (Modified) | U.S. Department of Commerce Patent and Trademark Office | Atty. Docket No. 28113/33072 | Serial No. 08/585,895 |
| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo and Joukov | |
| | | Filing Date 01/12/96 | Group 1806 1801 |

| OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.) | | |
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| | | Filing Date 01/12/96 | Group 1806 1801 |

| OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.) | | |
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| INFORMATION DISCLOSURE STATEMENT <i>(Use several sheets if necessary)</i> | | Applicant Alitalo et al. | |
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U.S. PATENT DOCUMENTS

| *Examiner Initials | Document Number | Issue Date | Name | Class | Subclass | Filing Date If Appropriate |
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FOREIGN PATENT DOCUMENTS

| *Examiner Initials | Document Number | Publication Date | Country | Class | Subclass | Translation | |
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| | | | | | | Yes | No |
| BW | B2 | WO 96/30046 | 10/03/96 | PET WO | — | | |
| BW | B3 | WO 95/33050 | 12/07/95 | PET WO | — | | |
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| INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary) | | Applicant Alitalo et al. | |
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| Full | B5 | WO 96/39421 | 12/12/96 | PCT WO | — | — | |
| Full | B6 | WO 96/39515 | 12/12/96 | PCT WO | — | — | |
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#11/15
01/16/97

PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

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) being deposited with the United
) States Postal Service as first class
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) date:

) Dated: November 26, 1997

) David A. Gass
) David A. Gass

AMENDMENT AND REPLY PURSUANT TO
37 C.F.R. §§ 1.111 AND 1.115

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In an official action mailed May 28, 1997, the U.S. Patent and Trademark Office (the Patent Office) rejected claims 1, 3-7, 11, 13, and 17-25 variously under 35 U.S.C. §§ 112, first and second paragraphs. The Applicants respectfully request reconsideration in light of the following amendments and remarks. This Amendment and Reply has been timely filed with a petition and fee for three months extension of time, extending the time period for response until November 28, 1997.

AMENDMENTS

In the specification:

Please amend the specification as follows:

At page 4, lines 14 and 21, delete "Flt-4" and substitute therefor --

Flt4 --

At page 5, line 14, delete "32" and substitute -- 33 --.

At page 5, line 17, delete "318" and substitute -- 317 --.

At page 6, line 31, delete "97321" and substitute therefor --

97231 --

At page 8, line 14, delete "Figure 5 shows" and substitute therefor

-- Figures 5A, 5B, and 5C show --.

At page 8, lines 19-20, delete "fractions from the Western analysis" and substitute therefor -- chromatographic fractions from the affinity purification --.

At page 8, please delete the brief description of Figure 10 at lines 30-32, and substitute therefor:

Figures 10A-10B show a comparison of the deduced amino acid sequences of PDGF-A (SEQ ID NO: 36); PDGF-B (SEQ ID NO: 37); two PIGF isoforms (SEQ ID NOs: 38 and 39); four VEGF isoforms (SEQ ID NOs: 40-43); and Flt4 ligand (VEGF-C) (SEQ ID NO: 33).--.

At page 9, line 5, after "lines" please insert -- and in brain tissue --.

At page 9, lines 6 and 10, delete "VEFG-C" and substitute therefor -- VEGF-C --.

At page 9, line 27, delete "its cloning" and substitute therefor -- the cloning of a DNA encoding this growth factor --.

At page 10, lines 1-2, delete "Claimed ligands" and substitute therefor -- Ligands of the invention --.

At page 11, line 7, after "residues" insert -- of --.

At page 11, line 19, delete "Balbaini" and substitute therefor -- Balbiani --.

At page 12, line 2, delete "have" and substitute therefor -- has --.
At page 12, line 7, delete "to structure to" and substitute therefor
-- in structure to --.

At page 12, line 23, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --.

At page 12, line 25, after "cells" insert -- (BCE) --.

At page 13, line 14, delete "the".

At page 13, line 17, delete "diseases" and substitute therefor --
diseases --.

At page 13, line 17, delete "to".

At page 14, line 4, delete "genes" and substitute therefor -- gene --.

At page 14, line 15, delete "these genes" and substitute therefor --
this gene --.

At page 14, line 20, delete "have" and substitute therefor -- has --.

At page 15, line 6, delete "the".

At page 15, line 12, after "Centricon 100" insert -- filters --.

At page 17, line 3, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --.

At page 17, lines 9 and 13, delete "analysed" and, in each
instance, substitute therefor -- analyzed --.

At page 17, line 13, after "50 μ g" insert -- of --.

At page 17, line 13, delete "was" and substitute therefor -- were --.

At page 17, line 18, delete "carboxyterminal" and substitute
therefor -- carboxy-terminal --.

At page 19, lines 26 and 28, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --.

At page 20, line 19, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --.

At page 20, line 30, delete "was" and substitute therefor -- were --

At page 21, line 16, delete "3" and substitute therefor -- 4 --

At page 21, line 27, delete "extracellular" and substitute therefor --
extracellular --

At page 22, line 5, delete "dialysed" and substitute therefor --
dialyzed --

At page 22, line 14, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --

At page 23, line 4, delete "Malborough" and substitute therefor --
Marlborough --

At page 23, line 19, delete "was" and substitute therefor -- were --

At page 24, line 21, delete "analysed" and substitute therefor --
analyzed --

At page 25, line 29, delete "analysed" and substitute therefor --
analyzed --

At page 27, line 17, delete "analysed" and substitute therefor --
analyzed --

At page 27, line 33, delete "32" and substitute therefor -- 33 --

At page 28, line 17, delete "NIH3T3" and substitute
therefor -- NIH 3T3 --

At page 28, line 19, delete "analysed" and substitute therefor --
analyzed --

At page 28, line 26, delete "slur" and substitute therefor -- slurry --

At page 29, line 1, after "97231.", please insert -- A 1997 base

C² pair nucleotide sequence of the cDNA insert of this deposited plasmid is set
forth in SEQ ID NOs: 44 and 45. --

At page 29, line 11, delete "two" and substitute therefor -- three --

At page 29, line 17, delete "Balbiani ring protein 3 (BRP3)" and substitute therefor -- Balbiani ring 3 protein (BR3P) --.

At page 29, line 22, delete "BRP3" and substitute therefor -- BR3P --.

At page 30, line 9, delete "assayed" and substitute therefor -- assayed --.

At page 30, line 11, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 30, line 34, delete "be" --.

At page 31, line 4, delete "analysed" and substitute therefor -- analyzed --.

At page 31, line 7, delete "10⁹" and substitute therefor -- 10⁹ --.

At page 31, line 23, delete "Metabolical" and substitute therefor -- metabolic --.

At page 31, line 30, delete "30 ml of a slur" and substitute therefor -- 30 μ l of a slurry --.

At page 32, line 3, delete "receptor binding" and substitute therefor -- receptor-binding --.

At page 32, line 24, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 32, line 34, delete "ml" and substitute therefor -- μ l --.

At page 33, line 3, delete "TBS" and substitute therefor -- RIPA --.

At page 33, lines 6, 14, and 25, delete "analysed" and, in each instance, substitute therefor -- analyzed --.

At page 33, line 19, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 33, line 31, after "(" please insert -- Fig. 14A --.

At page 34, line 10, delete "analysed" and substitute therefor -- analyzed --.

At page 34, line 14, delete "NIH3T3" and substitute therefor -- NIH 3T3 --.

At page 36, line 20, delete "highly expressed in all tissues analysed" and substitute therefor -- highly expressed in all tissues analyzed --.

At page 37, line 19, delete "Gaithersburg" and substitute therefor -- Gaithersburg --.

At page 38, line 3, delete "analysed" and substitute therefor -- analyzed --.

At page 38, line 12, delete "VEGF-B" and substitute therefor -- VEGF-C --.

At page 38, line 27, delete "c6" and substitute therefor -- C6 --.

At page 39, line 16, delete "20952" and substitute therefor -- 20852 --.

Please delete pages 40-49 of the specification, which comprise the original sequence listing, and substitute therefor new pages 40-60 filed herewith, which constitute a substitute Sequence Listing. In view of this amendment, please renumber the pages of claims and abstract beginning with "61" (to preserve consecutive page numbering).

In the claims:

Please cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38 as shown below:

1. (Twice amended) A host cell transformed or transfected with a (purified and isolated) polynucleotide encoding a polypeptide [which specifically binds] that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

C4
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wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

Sub
D6
C5
3. (Twice amended) A host cell transformed or transfected with a [purified and isolated] nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

4. (Amended) A host cell [The nucleic acid] according to claim 3 wherein said nucleic acid comprises [having] the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33 [the nucleic acid according to claim 3].

Sub D7
C6
7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P) [the vector according to claim 6].

C7
11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises [approximately] amino acids 1 to 120 of SEQ ID NO: 33.

Sub D8
C8
18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33[, said portion encoding a polypeptide capable of binding to an Flt4 receptor tyrosine kinase] effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

C9
20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 [kd] kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

-- 26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

C10
Sub D10
27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

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cont.
31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight

conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

C¹⁰
word.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell. --

REMARKS

I. History of claims and explanation of amendments.

A. Prosecution History

The application as filed contained 16 claims.

In an official communication dated November 25, 1996, claims 1-16 were subjected to a restriction requirement. In an Amendment and Election in Response to Restriction Requirement filed on January 24, 1997, the Applicants: elected claims directed to nucleic acids, vectors, and host cells; canceled claims 2, 8-10, 12, and 14-16; amended claims 1, 3, 5, 11, and 13; and added claims 17-25.

In the outstanding Office action dated May 28, 1997, claims 1-3, 7, 11, 13, 17-25 were rejected. In the present amendment, the Applicants cancel claims 6, 13, and 17; amend claims 1, 3-5, 7, 11, 18, and 20; and add new claims 26-38. Thus, claims 1, 3-5, 7, 11 and 18-38 are now pending. A copy of the claims, in their amended forms, is appended hereto for the Examiner's convenience.

The Applicants do not intend by the amendments herein or any other amendments to abandon the subject matter of any claim as originally filed or as previously amended, and reserve the right to pursue such subject matter in subsequent applications, such as continuations, continuations-in-part, and divisional applications.

B. Amendments to the specification.

Most of the amendments to the specification correct obvious typographical errors, grammatical errors, and the like.

The amendments at page 5, lines 14 and 17, correct obvious typographical errors in the designation of the portions of SEQ ID NO: 33 which correspond to the unprocessed and "mature" forms of VEGF-C. The description of the amino terminus of a mature form of VEGF-C is found in the specification at, e.g., p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26,

line 6 (from which it is apparent that the first 13 amino acid residues of a secreted Flt4 ligand are encoded by the thirty-nine 3' bases of SEQ NO: 25 that begin ACAGAAGAGACT...). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered. As explained in the accompanying statement, a corrected SEQ ID NO: 33 has been filed herewith.

The amendment at page 6 to correct the ATCC accession number corrects an obvious typographical error, as is apparent from the ATCC deposit information provided at page 39, lines 14-18.

The amendment to the description of Figure 7 at page 8, lines 19-20; finds support at pages 21-22 (Example 5), from which it is apparent that Figure 7 depicts the results of gel electrophoresis of chromatographic fractions from the affinity purification of the Flt4 ligand.

The description for Figure 10 has been amended to reflect that Figure 10 is presently two panels (Figs. 10A-10B) and to include cross-references to the amended sequence listing. These amendments are responsive to objections raised in paragraphs 5 and 6 of the Office action.

The amendment at page 9, line 5, to add "brain tissue" to the description of Figure 11 finds support in Figure 11 itself, wherein the gel lane depicting the results for brain tissue is clearly labeled.

The amendments at pages 11 and 29 to correctly designate "Balbiani ring 3 protein" find support in the articles referenced at page 11, and the correct designations would have been understood by one skilled in the art.

The corrected cross-reference to Example 4 at page 21, line 16, finds support in Example 4 itself, because it is readily apparent from reading the application that Example 4 characterizes the ligand expressed by PC-3 cells.

The amendment at page 27, line 33, finds support as described above for the similar amendment at page 5, line 14.

The amendments to add SEQ ID NOs: 44-45 and include a cross-reference thereto at page 29, line 1, find support in the deposited plasmid

pFLT4-L, as an inherent property of the plasmid. See *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); *Therma-Tru Corp v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276 (Fed. Cir. 1995) ("[T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); and *Kennecott Corp. v. Kyocera International Inc.*, 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date).

The amendment at page 29, line 11 finds support in Figure 10, wherein three (not two) putative N-linked glycosylation sites are underlined.

The substitution of " μ " for "ml" at page 32, line 33, would have been obvious to the person of ordinary skill in the art from the context of Example 14, due to the nature of the experiment.

The corrected designation of a wash buffer used in the VEGFR-2 binding experiments at page 33, line 3 (Example 14), improves the readability of the eventual patent. This correction does not relate to how one would make or use the subject matter of the claims.

The amendment at page 33, line 31, to provide a cross-reference to Fig. 14A finds support in the context of the discussion of Figs. 14A-14B at page 33 and in the figures themselves, and will improve the readability of the eventual patent.

Support for the amendment at page 38, line 12, to substitute "VEGF-C" for "VEGF-B" is apparent from the context at page 38, lines 10-18, from which it is apparent that VEGF-C-encoding DNA is being discussed.

Support for the substitute Sequence Listing filed herewith is provided in the accompanying statement filed herewith.

C. Amendments to the claims.

All of the claim amendments find support throughout the application as originally filed.

For example, the recitations in claim 1 relating to binding to the extracellular domain of human Flt4 find support at p. 5, lines 4-9; p. 9, lines 30-32; p. 10, lines 26-31; p. 14, lines 30-34; and Examples 4 and 5.

The hybridization conditions recited in claim 1 find support in Example 10 (especially at p. 27, lines 9-14), wherein the recited hybridization conditions were employed in the isolation of VEGF-C-encoding cDNAs from a cDNA library.

The recitations in claims 1, 3, 7, 18, and 30 regarding expression of a polypeptide that includes a VEGF-homologous domain but excludes any domain having homology to a Balbiani ring 3 protein finds support in Example 13 (teaching that transfected host cells express and secrete 32 kD and 23 kD forms of VEGF-C that bind Flt4); at p. 11, lines 11-23 (teaching that the 23 kD polypeptide is likely to represent the VEGF-homologous domain, and that the carboxy-terminal amino acid sequences that show a cysteine pattern reminiscent of the Balbiani ring 3 protein is at least partially cleaved off); at page 11, lines 33-35, and in Fig. 10 (describing and depicting the eight conserved cysteine residues of the PDGF/PIGF/VEGF family of proteins).¹

The recitation of plasmid pFLT4-L in claim 7 finds support in claim 6 (from which claim 7 originally depended).

The amendment of claim 20 to substitute "kD" for "kd" is not intended as a substantive change, but merely is intended to increase uniformity of the eventual patent.

The recitation of an expression vector in claim 29 finds support in Example 11, e.g., at p. 28, lines 5-13; and at p. 6, lines 27-31.

The amino acid ranges recited in claims 31 and 33 find support at page 5, lines 27-33.

¹ In Fig. 10, the eight conserved cysteines are readily apparent at positions 103, 130, 136, 139, 140, 147, 184, and 186. In SEQ ID NO: 33 of the amended sequence listing, these eight cysteines correspond to residues 29, 54, 60, 63-64, 71, 107, and 109.

Support for claims 36-38, directed to a method for producing a polypeptide with host cells of the invention, is found at p. 6, lines 32-35, for example.

II. The second inventors' declaration, filed August 12, 1996, is not defective.

In paragraph 2 of the Office action, the Patent Office alleged that the inventors' declaration was defective due to non-initialed alterations and failure to acknowledge that the application is a CIP. The alleged defects are rendered moot by the second inventors' declaration that accompanied the Applicants' preliminary amendment dated August 12, 1996. Copies of the amendment and declaration are attached hereto as Exhibits 1 and 2.

III. Proposed drawing correction.

In paragraphs 3-4 and 6 of the Office action, the Patent Office requested the submission of a proposed drawing correction and the amendment of the brief description of the drawing to identify the two pages of Figure 10 as "10A and "10B". Attached hereto as Exhibit 3 is a proposed (informal) drawing correction. The Brief description of the drawing has been appropriately amended as well, at page 8, line 32. Accordingly, these objections may now be withdrawn. The Applicants wish to defer formal correction of the drawings and submission of a petition for photograph drawings until the application is allowed.

IV. The Application is in compliance with the Sequence Rules.

In paragraph 5 of the Office action and in a Notice to Comply, the Patent Office requested that the Application be amended to include the Figure 10 sequences therein, and to include cross-references to the Sequence Listing in the brief descriptions of Figs. 9B and 10. The Application has been so amended. The sequence listing amendment is accompanied by an appropriate

statement confirming that no new matter has been introduced into the application. Accordingly, this objection may properly be withdrawn.

V. The Applicants request deferral of the requirement for a Budapest Treaty declaration.

In paragraph 7 of the Office action, the Patent Office rejected claims 6 and 7 under 35 U.S.C. § 112, first paragraph, alleging that access to biological deposit material was required to use the claimed invention. As indicated in the specification at p. 39, the biological deposit was made pursuant to the provisions of the Budapest Treaty. A statement confirming the availability of the deposit is filed herewith, rendering this rejection moot.

The Budapest Treaty declaration filed herewith is intended solely to expedite prosecution, and is not intended as an admission that the deposited plasmid is required to satisfy § 112, first paragraph.

VI. The rejection of claim 1 under § 112, first paragraph, should be withdrawn.

In paragraph 8 of the Office action, the Patent Office rejected claim 1, alleging that the full scope of the claim was not enabled by the specification:

Claim 1 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for polynucleotides [sic: encoding?] all polypeptides that bind the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

The scope of claim 1 encompasses polynucleotides from any source that encode polypeptides that bind specifically to the Flt4 receptor. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the encoded proteins

are recited in the instant claims. There is no guidance provided by the specification to select those encompassed polynucleotides that encode proteins that specifically bind the Flt4 receptor with the exception of those teachings which support the subject matter indicated as enabled. There is no guidance to predict *a priori* whether any protein would bind the receptor *without some information on the structure of the protein*, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention.

(Office action at p. 5.)

The Applicants traverse-in-part and amend-in-part.

A. The amendments to claim 1 overcome the factual bases for the Patent Office's rejection.

Claim 1 has been amended such that it no longer encompasses every polynucleotide that encodes all polypeptides that bind the Flt4 receptor. The Applicants have adopted the Examiner's suggestion to include additional limitations relating to the structure of the encoded protein.

In particular, amended claim 1 is directed only to polynucleotides that encode polypeptides that: (1) have a VEGF-homologous domain defined by eight conserved cysteines that are common to the PIGF/PDGF/VEGF family of polypeptides (see Fig. 10, positions 103, 130, 136, 139, 140, 147, 184, and 186); and (2) are capable of binding with *high affinity* to the *extracellular domain of human Flt4*. Thus, claim 1 has been further limited with respect to the source (human) and the domain (extracellular) of the binding partner; the nature (high affinity) of the binding reaction; and the type of polypeptide encoded (polypeptides which possess homology to a core portion of VEGF that is definable by eight conserved cysteines).

In addition, claim 1 now contains a significant structural limitation relating to the sequence of the claimed polynucleotide, namely, that the polynucleotide is sufficiently similar to the exemplified SEQ ID NO: 32 such that it will hybridize to the non-coding strand complementary to SEQ ID NO: 32 under specified hybridization conditions. The specified hybridization conditions

are those that were successfully employed in Example 10 to screen a PC-3 cell cDNA library to clone VEGF-C cDNA. (See specification at pp. 26-27.)

The scope of amended claim 1 is commensurate with the teachings in the application. Because of the hybridizing limitation, claim 1 reads only on those polynucleotides that can be identified via a routine hybridization screening assay that has been taught and successfully performed in the present application. Moreover, there is guidance in the specification that Flt4 ligands of the invention contain homology to VEGF (see, e.g., specification at p. 11, lines 11-13, and Figs. 10A-10B), and claim 1 has been appropriately limited in this manner. The specification teaches Flt4 binding assays that are useful to determine whether an encoded polypeptide binds Flt4.

In fact, subsequent hybridization experiments, using the human VEGF-C cDNA as a probe, were successfully performed to isolate VEGF-C-encoding cDNAs of mouse and quail. (See Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo at ¶¶ 10-18.) The identity of the encoded proteins was confirmed by receptor binding and stimulation studies. (*Id.*) This evidence that the specification enables one to isolate non-human VEGF-C-encoding cDNAs and confirm their identity in the receptor binding studies refutes the basis for the Patent Office's rejection.

For all of these reasons, claim 1 as amended is commensurate in scope with the teachings in the application, and the rejection under §112, first paragraph, should be withdrawn.

B. The Borg reference cited by the Patent Office does not support the rejection.

The Patent Office also cited a Borg publication in support of its rejection:

There is no guidance provided by the state of the art to select ligands to make the invention; despite intense research in this area, Borg et al. (reference C7) teach that no known ligands for the Flt4 receptor were known at the time of the invention.

(Office action at pp. 5-6.)

Reliance upon Borg is misplaced, because the Applicants do not rely upon Borg to provide an enabling disclosure. The Applicants specification provides the enabling disclosure. As explained above, amended claim 1 is commensurate in scope with guidance provided in the specification.

- C. The legal authorities relied upon by the Patent Office do not support a rejection of claim 1.

The Patent Office cites several cases in support of its rejection of claim 1:

The amount of guidance required varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). See also *Genentech, Inc. v. Novo Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at p. 6.)

The *Soll* opinion relied upon by the Patent Office is distinguishable because the patent applicant in that case was attempting to claim more broadly than the original disclosure of the patent application, which disclosure gave no indication that the applicant regarded his invention as a generic one. See *In re Soll*, 38 U.S.P.Q. at 190. In the present case, claim 1 has been amended herein to claim more narrowly than the generic invention originally contemplated and claimed by the inventors.

The *Fisher* opinion relied upon by the Patent Office relates to patent applications filed in the 1949-1960 period, well in advance of the genetic engineering techniques that were available and known in the art at the time the present application was filed. See *In re Fisher*, 166 U.S.P.Q. at 19-20. Because the genetic engineering techniques known to those skilled in the art at the time of the present application drastically reduce experimentation relative to

the traditional techniques that were known at the time of *Fisher*, the *Fisher* opinion cannot properly be applied against the present case.

The Patent Office's reliance upon the *Genentech* decision also is misplaced, because the facts of that case are wholly dissimilar from the facts of the present case.

Initially, it should be observed that *Genentech* is a case wherein the Court analyzed whether a 1979 patent application satisfied the enabling disclosure requirement. See *Genentech Inc. v. Novo Nordisk A/S*, 42 U.S.P.Q.2d 1001, 1004 (Fed. Cir. 1997). The state of the art relating to recombinant DNA and proteins was in a state of relative infancy in 1979, as compared to the state of the art in the 1994-1996 time period during which the present series of applications were filed. Since the enabling disclosure requirement involves an analysis of whether an application enables one of ordinary skill in the art to make and use an invention, and since the skill in the art has advanced enormously since 1979, the *Genentech* opinion is of little relevance.

Moreover, *Genentech* involved a unique situation wherein a patentee re-filed a patent application with a wholly new claim, in an attempt to enjoin an alleged infringer. As characterized by the Federal Circuit, the unique factual circumstances were as follows: an unsolved problem in the art had been the difficulty of obtaining a human protein (hGH) from a precursor that contained added protein material; Genentech's specification taught a solution to the problem wherein hGH was expressed *without* the added material; yet Genentech was attempting, in its re-filed application, to "bootstrap" by claiming a wholly different solution to the problem, namely, expression via synthesis and processing of a cleavable fusion protein. *Genentech*, 42 U.S.P.Q.2d at 1005. Genentech's specification contained "no disclosure of any specific starting material or of any of the conditions under which [the claimed] process can be carried out." *Id.* In these circumstances, the Court found Genentech's patent invalid.

In contrast, the present application teaches the procedures necessary to identify polynucleotides according to claim 1. For example, the present application teaches hybridization assays to identify candidate polynucleotides from other cDNA libraries; expression techniques for expressing polypeptides; and screening techniques to identify those polypeptides that bind to the extracellular domain of human Flt4.

The final case relied upon by the Patent Office, *In re Wands*, actually *supports*, rather than negates, a conclusion of enablement. The *Wands* opinion stands for the proposition that "Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). In *Wands*, the Federal Circuit reversed as improper a rejection under §112, first paragraph. The Court recognized that practitioners in the pertinent molecular biological art were prepared to perform multiple screening experiments of "negatives" in order to identify one "positive." *Wands*, 8 U.S.P.Q.2d at 1406. Moreover, for the purposes of evaluating whether experimentation is "undue," the Federal Circuit recognized that "an experiment" was not simply defined by the screening of a single clone, but rather, by a larger process that can involve producing and screening several clones:

Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics.

In re Wands, 8 U.S.P.Q.2d at 1407.

As explained more fully in Section VII, below, the present specification enables the present claims, under the standards established in *Wands*.

VII. The rejection of claims 18-25 under §112, first paragraph, should be withdrawn.

In paragraph 9 of the Office action, the Patent Office rejected claims 18-25, alleging that the full scope of the claims was not enabled by the specification:

Claims 18-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for polynucleotides having the sequence set forth in SEQ ID NO:32, for polynucleotides encoding polypeptides having the amino acid sequence set forth in SEQ ID NO:33, and for polypeptides comprising residues 1-120 or 1-180 of SEQ ID NO:33, does not reasonably provide enablement for the scope of polynucleotides commensurate with the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at pp. 6-8.)

The Applicants traverse-in-part and amend-in-part.

A principal basis for the rejection relates to the alleged scope of encoded-polypeptides encompassed by the claims:

The breadth of claims 18-25 encompasses polynucleotides encoding all active fragments of the protein of SEQ ID NO:33, or those that are 23 kDa or 32 kDa in size. Claim 21 recites the limitation that the fragment comprises SEQ ID NO:13, but this claim encompasses polypeptides in which SEQ ID NO:13 is the only sequence derived from the protein of SEQ ID NO:33. The only guidance offered by the specification to those fragments that may make the invention is provided at page 11 where at least residues 1-120 of SEQ ID NO:33 are taught to be required for activity by comparison to PDGF (*infra*).

(Office action at pp. 6-7.)

The Applicants respectfully submit that the breadth of the claims are commensurate in scope with the guidance in the specification.

- A. There is no undue experimentation involved in synthesizing the polynucleotides (or the encoded polypeptides) within the scope of the claims.**

By providing the amino acid sequence set forth in SEQ ID NO: 33, the specification enables one skilled in the art to make essentially any polypeptide comprising a portion of SEQ ID NO: 33, and enables one to make essentially any polynucleotide coding sequence for such polypeptide. For example, such polynucleotides may be synthesized using automated synthesizers or using recombinant techniques (e.g., using polynucleotides of the invention and/or variants thereof obtained by site-directed mutagenesis).

- B. There is no undue experimentation involved in screening polypeptides for the abilities to bind Flt4 or stimulate Flt4 phosphorylation.**

Claim 18 is limited to nucleic acids that comprise a sequence encoding a polypeptide comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4. This limitation is commensurate in scope with the teachings in the application, because the specification teaches that encoded polypeptides are Flt4 ligands, and teaches Flt4 binding assays (and phosphorylation assays) to determine whether a polypeptide is capable of binding to Flt4 receptor tyrosine kinase (and whether the peptide is capable of stimulating Flt4 autophosphorylation). (See, e.g., Examples 4-5.) Such assays are the "routine screening" type of assay contemplated by the Federal Circuit in the *Wands* opinion.

- C. The specification provides guidance for identifying portions of SEQ ID NO: 33 effective to permit Flt4 binding.**

The specification provides significant guidance for determining portions of SEQ ID NO: 33 that are effective to permit Flt4 binding. For example, although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the first 33 amino acids are not critical for Flt4 binding. (See, e.g., p. 23, lines 5-10, and p. 25, line 27, to p. 26, line 6, which teach that a mature form of VEGF-C lacks the first 33 residues of SEQ ID NO: 33.)

The specification further teaches that the amino acids essential for retaining Flt4 ligand activity are contained within approximately amino acids 1-120 of SEQ ID NO: 33, and that the proteolytic cleavage that produces a mature, naturally occurring Flt4 ligand occurs within approximately amino acids 1-180 of SEQ ID NO: 33. (Specification at p. 5, lines 27-31.) There is guidance that the observed ~23 kD polypeptide exemplified in the application is likely to represent the VEGF-homologous domain of VEGF-C, and that the carboxy-terminal sequences that contain cysteine motifs reminiscent of a Balbiani ring 3 protein are cleaved off. (Specification at p. 11, lines 4-23.) At page 11, lines 33-35, attention is drawn to the probable importance of eight conserved cysteine residues of VEGF-C, which correspond to residues 29, 54, 60, 63-64, 71, 107, and 109 of SEQ ID NO: 33. (See Figure 10 and the amended Sequence Listing filed herewith.)

Additionally, the specification outlines a protocol for defining that portion of SEQ ID NO: 33 which corresponds with the naturally-occurring Flt4 ligand. (See pp. 29-30.) Furthermore, the specification provides guidance to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. (Specification at p. 30, lines 6-17.) The Declaration Under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith provides evidence that such procedures were successful in further characterizing the natural processing of VEGF-C and in identifying VEGF-C fragments that are capable of binding Flt4. (See ¶¶ 6-9.)

Collectively, these teachings serve to both provide guidance for predicting the portions of SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.

D. The Patent Office's reliance upon Heldin to support its rejection is improper.

The Patent Office cited a Heldin publication in support of its rejection:

Heldin et al. (reference C40), however, teach the criticality of structures throughout the corresponding region of PDGF, such as extended loop structures (Figure 2), disulfide bonds involving residue 1 (page 249, column 1), and specific residues including those up to residue 154 (*loc. cit.*). Moreover, comparison with PDGF is of limited predictive value, because the stereo-specific interaction required between VEGF-C and its receptor are different than those between PDGF and its receptor as evidenced by the fact that the two ligands do not bind the same receptors. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Recitation that the encoded proteins must be 23 or 32 kDa in size does not provide significant additional guidance or limitation to the scope of the claims, because this limitation does not exclude the presence of sequences unrelated to VEGF-C within the polypeptide, nor does it exclude various post-translational modifications known to profoundly influence the apparent molecular weight without affecting the primary sequence of the polypeptide. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. In re Fisher, 166 USPQ 18 (CCPA 1970).

(Office action at p. 7.)

The Applicants agree that comparison with PDGF, *by itself*, would be of limited predictive value. However, as outlined above in Section C, comparison with PDGF is merely one of many factors taught in the specification for predicting which fragments bind the receptor; and the application teaches routine screening to determine which fragments *actually* bind the receptor.

- E. The Patent Office's suggestion that one skilled in the art must test every fragment of SEQ ID NO: 33 is incorrect.

Underlying the Patent Office's rejection is the implicit assumption that the experimentation involved to practice the invention would require the screening of every possible fragment of SEQ ID NO: 33:

The vast amount of experimentation required to test all the encompassed fragments is an additional factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

(Office action at pp. 7-8.)

As an initial matter, the Applicants wish to clarify that the claims as written are directed to polynucleotides comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4. The "testing" of polypeptide fragments for Flt4 binding determines whether polynucleotides encoding the fragments fall within the claims. Therefore, to the extent the claims have been interpreted as "encompassing" both binding and non-binding fragments, the Patent Office has improperly ignored a limitation of the claims.

Second, the Patent Office's assumption that one must test "all" fragments improperly ignores the significant guidance in the specification with respect to those portions of SEQ ID NO: 33 that are effective to permit binding to Flt4. This guidance drastically reduces the number of fragments that one would select for screening.

Moreover, the Patent Office's suggestion that it is necessary to test all fragments of SEQ ID NO: 33 ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that portion of SEQ ID NO: 33 that is effective for binding, and

that portion which is not.² An artisan of ordinary skill also understands techniques for accelerating a screening process,³ and techniques for screening multiple polypeptides *simultaneously*. Thus, the examiner's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

F. The basis for rejection is moot with respect to the Applicants new claims 30-36.

New claims 30-36 contain additional limitations (relative to rejected claims 18-25) that characterize portions of SEQ ID NO: 33 which are effective to permit Flt4 binding, and which are encoded by the claimed polynucleotide. These additional limitations render inapplicable the bases for rejection that the Patent Office alleged against claims 18-25. Evidence in support of the patentability of these claims is provided in paragraphs 6-9 of the Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo filed herewith.

VIII. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.

In paragraph 10 of the Office action, the Patent Office rejected claims 3-5, 11, 13, and 17-25 under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention.

² For example, a determination that a polypeptide comprising residues 1-120 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 121-317 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

³ For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 1-100, 1-110, 1-120, 1-130, 10-120, 20-120, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (1-130, 1-129, 1-128, 1-127, 1-126 . . .), to more rapidly identify effective portions for binding Flt4.

The Patent Office relied upon a Human Genome Sciences publication in support of its rejection:

SEQ ID NO:32 encodes a protein whose amino terminus is indicated as residue 1, and the specification teaches that the amino terminal of a 23 kDa protein expressed from the polynucleotide has the amino terminus shown in SEQ ID NO:13; however, Human Genome Sciences, Inc. disclose DNA encoding a similar sequence (99% global identity using the Smith-Waterman algorithm with 100% identity to the instantly claimed protein in the amino terminus through the instant residue 8) whose amino terminus is indicated as residue -8 of the instant protein. It would have been understood in the art that a disclosure of a particular residue as "residue 1" would have meant that this residue was the amino terminus of the mature polypeptide; however, it was unclear which residue corresponds to the amino terminus of the encoded polypeptide, making the designation of a particular amino acid residue as a "residue 1" indefinite. Although Human Genome Sciences was published after the effective filing date, the publication is used to show that the instant claims were indefinite at the time of filing. MPEP 2124 citing *In re Glass*, 492 F.2d 1228, 1232 n. 6., 181 USPQ 31, 34 n.6 (CCPA 1974).

(Office action at p. 8.)

Clarification is in order.

- A. The present application teaches that amino acid 1 is the threonine that is the 34th residue of SEQ ID NO: 33.

The Patent Office is correct that the specification teaches that the amino terminal amino acid of a 23 kDa protein expressed from a polynucleotide comprising SEQ ID NO: 32 has the amino terminus shown in SEQ ID NO: 13. This amino terminus corresponds with the 34th residue in SEQ ID NO: 33. As explained in the Statement Pursuant to 37 C.F.R. §1.825 filed herewith, the Sequence Listing has been amended herein to reflect the fact that this threonine residue represents the amino terminus of a mature VEGF-C protein.

The amino terminus taught in the present application reflects the results of amino acid sequencing of a purified VEGF-C protein secreted from a human cell line. (See Specification at pp. 21-23 (Example 5).) Thus, the

Applicants' asserted amino terminus is based upon the scientific characterization of a secreted human protein.

- B. Human Genome Sciences did not establish the amino terminus of a mature VEGF-C protein, and the cited publication is a mere guess that Human Genome Sciences later withdrew.

Apparently, the Patent Office relies upon Human Genome Science's International Patent Publication WO 95/24473 (hereinafter "HGS1") in support of its rejection. As set forth below, the HGS1 publication contains no sound scientific data to render indefinite the present claims or to call into question the Applicants' determination of the correct amino terminus of a mature VEGF-C protein.

The HGS1 publication teaches a "VEGF2" polypeptide "comprising 350 amino acids residues of which *approximately* the first 24 amino acids represent the leader sequence." (HGS1 at p. 4 (emphasis added).) The HGS1 publication does not base its determination of a "mature" 326 amino acid polypeptide on any scientific data. In fact, the only purported expression studies in the HGS1 publication were *in vitro* expression of PCR-amplified portions of cDNAs. (HGS1 at pp. 28-29.) The *in vitro* expression machinery employed would not necessarily process the expressed protein, and the HGS authors do not even report any analysis of the amino terminus of this *in vitro* protein. Because the splice site in the HGS1 publication is pure speculation, the authors assert only that "the first 24 amino acids residues are *likely to be leader sequence* (HGS1 at p. 5 (emphasis added).)"⁴

In fact, International Patent Publication No. WO 96/39515 (hereinafter "HGS2"), a later publication by a different Human Genome Sciences authorship entity, seems to *refute* the definition of a "mature" amino terminus that is proffered in HGS1. More particularly, HGS2 alleges that "VEGF2 contains an open reading frame encoding a protein of 419 amino acid residues

⁴ Moreover, HGS1 fails to identify a VEGF2 binding partner and fails to demonstrate a VEGF2 biological activity.

of which approximately the first 23 amino acid residues are the putative leader sequence such that the mature protein comprises 396 amino acids...." (HGS2 at p. 7.) Thus, more than one year after HGS1, the Human Genome Sciences scientists have apparently retracted or contradicted the teachings in HGS1 relating to a proper definition of "mature" VEGF2. HSG2, like HGS1, contains no scientific study to define a true "mature" VEGF2.

Thus, a careful scientific analysis reveals that the present application properly defines an amino terminus for a mature VEGF-C protein, based on sequencing studies of a VEGF-C protein that is actually expressed in a human cell line. The multiple, different amino termini alleged by Human Genome Sciences in its publications are mere speculation, unsupported by scientific evidence. Because the Applicants' asserted amino terminus is scientifically supported and the Human Genome Sciences purported amino termini are mere speculation, the Patent Office's indefiniteness rejection of claims 3-5, 11, 13, and 17-25 should be withdrawn.

IX. The rejection of claims 3-5, 11, 13, and 17-25 under §112, second paragraph, should be withdrawn.

In paragraph 11 of the Office action, the Patent Office rejected claims 11 and 13, under 35 U.S.C. §112, second paragraph, alleging that these claims were indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention:

Specifically, the term "approximately" in claims 11 and 13 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

(Office action at p. 9.)

Solely for the purpose of expediting allowance, the Applicants have amended claim 11 to remove the allegedly indefinite term. Claim 13 has been canceled

herein. These amendments render this rejection moot. The rejection should therefore be withdrawn.

X. Comments concerning the Examiner's statement of reasons for the indication of allowable subject matter.

In paragraph 13 of the Office action, the Examiner commented as follows about the state of the art:

The following is a statement of reasons for the indication of allowable subject matter: polynucleotides encoding the instantly claimed ligand appear to be novel over the prior art of record. Borg et al. (reference C7), for example, disclose that the ligand for Flt4 was not known in the art around the time of invention. Closest prior art is a DNA with about 99% identity to the claimed polynucleotide (Human Genome Sciences, Inc. reference B1), but the publication date antedates the effective filing date of the instant application. Other relevant prior art made of record below discloses a series of expressed sequence tags (ESTs) with high identity to large regions of the Flt4 ligand cDNA. The probable identity of these ESTs was not disclosed, and without the benefit of hindsight, the artisan at the time of invention would not have been motivated to use these ESTs to make the claimed invention. It was not known that these ESTs encoded a receptor ligand, nor was it known that these ESTs were nearly identical to the Flt4 ligand cDNA at the time of invention. In fact, the only EST posited to encode a particular protein was taught to encode a Balbiani ring protein (Hillier et al., EST-STS Accession No. T81690), hardly giving motivation to use the EST to find the claimed invention.

(Office action at pp. 9-10.)

The Applicants first wish to clarify that the publication date of reference B1 does *not* antedate the effective filing date of the instant application. It is apparent from the context of the Office action that this is what the Examiner intended.

Moreover, the Applicants respectfully submit that all of the claims in the present application would be patentable over reference B1, even if that reference (or a counterpart U.S. patent) constituted statutory prior art.

For example, claim 1 is directed to a host cell transformed or transfected with a polynucleotide of the invention, wherein the host cell expresses a polypeptide encoded by said polynucleotide, *said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P)*. Reference B1 does not even suggest to try to recombinantly express a polypeptide lacking domains having BR3P cysteine motifs. Even if such a suggestion existed, there would be no reasonable expectation from reference B1 that such a polypeptide would bind Flt4. In fact, there is no recognition in reference B1 that any polypeptide binds Flt4. Thus, the subject matter of claim 1 is novel and unobvious over reference B1. It will be apparent that similar reasoning supports the novelty and nonobviousness of host cell claims 3, 4, 5, 7, 25-29, and 35, and of method claims 35-38.

Claim 18 is directed to a nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to Flt4, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, *said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein*. It is totally unexpected from reference B1 that a nucleic acid that encodes a portion of SEQ ID NO: 33 that lacks the recited BR3P encoding sequences still encodes a polypeptide that is capable of binding Flt4. These unexpected properties support the unobviousness of claim 18 and claims that depend therefrom. Similar considerations support the unobviousness of claims 33-35 over reference B1.

Thus, all of the pending claims would remain patentable over reference B1, even if this reference were statutory prior art.

XI. Prosecution has been suspended in a related application.

Pursuant to 37 C.F.R. §1.56, the Applicants wish to apprise the Examiner that prosecution has been suspended in a parent application (U.S.S.N. 08/510,133) "because a reference relevant to the examination . . . may soon become available." As set forth in Section X, above, if the reference is a U.S. patent counterpart to reference B1, the reference should not prevent allowance of the present application. All of the pending claims are directed to subject matter that is patentably distinct from anything disclosed or suggested in reference B1.

CONCLUSION

For the foregoing reasons, the applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification, and allowance of claims 1, 3-5, 7, 11, and 18-38.

Respectfully submitted,

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Date: Nov. 26, 1997

APPENDIX OF CLAIMS

1. (Twice amended) A host cell transformed or transfected with a polynucleotide encoding a polypeptide that is capable of binding with high affinity to the extracellular domain of human Flt4 receptor tyrosine kinase, wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(a) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(b) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS; and

wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

2. [CANCELED]

3. (Twice amended) A host cell transformed or transfected with a nucleic acid encoding a polypeptide having the amino acid sequence shown in SEQ ID NO: 33, wherein said host cell expresses a polypeptide encoded by said polynucleotide, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

4. (Amended) A host cell according to claim 3 wherein said nucleic acid comprises the sequence shown in SEQ ID NO: 32.

5. (Twice amended) A host cell according to claim 3 wherein said polynucleotide is a vector comprising a nucleic acid that encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 33.

6. [CANCELED]

7. (Amended) A host cell comprising plasmid pFLT4-L, deposited as ATCC accession No. 97231, wherein said host cell expresses a polypeptide encoded by said plasmid, said polypeptide including a domain defined by eight conserved cysteines having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P).

8. [CANCELED]

9. [CANCELED]

10. [CANCELED]

11. (Twice amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises amino acids 1 to 120 of SEQ ID NO: 33.

12. [CANCELED]

13. [CANCELED]

14. [CANCELED]

15. [CANCELED]

16. [CANCELED]

17. [CANCELED]

18. (Amended) A purified and isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide that is capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide having an amino acid sequence comprising a portion of the amino acid sequence shown in SEQ ID NO: 33 effective to permit such binding, said polynucleotide lacking a nucleotide sequence that encodes the portion of the amino acid sequence shown in SEQ ID NO: 33 that has cysteine motifs of a Balbiani ring 3 protein.

19. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase.

20. (Amended) A purified and isolated nucleic acid according to claim 19 wherein said polypeptide has an apparent molecular weight of about 23 kD as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

21. A purified and isolated nucleic acid according to claim 19 wherein said polypeptide comprises an amino-terminal amino acid sequence set forth in SEQ ID NO: 13.

22. A purified and isolated nucleic acid according to claim 21 wherein said polypeptide comprises approximately 120 amino acids.

23. A purified and isolated nucleic acid according to claim 18 wherein said polypeptide has an apparent molecular weight of about 32 kDa as assessed by SDS polyacrylamide gel electrophoresis under reducing conditions.

24. A vector comprising a nucleic acid according to claim 18.

25. A host cell transformed or transfected with a vector according to claim 24.

26. A host cell according to claim 1 that expresses a naturally occurring VEGF-C protein encoded by said polynucleotide.

27. A host cell according to claim 1 that expresses a human VEGF-C protein encoded by said polynucleotide.

28. A host cell according to claim 27, wherein said host cell expresses said polynucleotide and produces a mature human VEGF-C protein having a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions.

29. A host cell according to claim 1 wherein said polynucleotide is an expression vector, said expression vector including an expression control sequence operatively linked to a nucleotide sequence that encodes said polypeptide.

30. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion that includes a VEGF-homologous portion of SEQ ID NO: 33 and excludes the portion of SEQ ID NO: 33 that contains cysteine motifs of a Balbiani ring 3 protein.

31. A polynucleotide according to claim 18 wherein said portion of the amino acid sequence shown in SEQ ID NO: 33 is a continuous portion having amino acid 1 of SEQ ID NO: 33 as its amino terminal residue, and having as its carboxy terminal residue an amino acid between residues 119 and 126 of SEQ ID NO: 33.

32. A purified and isolated nucleic acid according to claim 19 wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

33. A polynucleotide encoding a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, said polypeptide consisting of a continuous portion of the sequence shown in SEQ

ID NO: 33, said continuous portion commencing at residue number 1 of SEQ ID NO: 33 and lacking at least carboxy terminal residues of SEQ ID NO: 33 beyond residue 125.

34. An expression construct comprising the polynucleotide according to claim 33 operatively linked to an expression control sequence.

35. A host cell transformed or transfected with the expression construct of claim 34.

36. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 35 under conditions which permit expression in said host cell of a polypeptide encoded by said polynucleotide; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

37. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to any one of claims 1, 3, 4, 5, 7, 26, or 27 under conditions which permit expression by said host cell of a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, said polypeptide including a domain defined by eight conserved cysteines and having homology to vascular endothelial growth factor (VEGF) and lacking any domain having cysteine motifs of a Balbiani ring 3 protein (BR3P); and

isolating said polypeptide from the host cell or the growth medium of the host cell.

38. A method for producing a polypeptide that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase, comprising the steps of:

growing a host cell according to claim 25 under conditions which permit expression by said host cell of a polypeptide encoded by said nucleic acid that is capable of binding the extracellular domain of human Flt4 receptor tyrosine kinase; and

isolating said polypeptide from the host cell or the growth medium of the host cell.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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- (ii) TITLE OF INVENTION: RECEPTOR LIGAND
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(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/585,895
(B) FILING DATE: 12-JAN-1996
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- C³
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(A) APPLICATION NUMBER: US 08/510,133
(B) FILING DATE: 01-AUG-1995
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/340,011
(B) FILING DATE: 14-NOV-1994
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(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG
GACTCCTGGA

60

70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCCGCCGGTC ATCC

24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCC CATGACCCCA AC

22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT

33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTAGGTGA CACTATA

17

C3
cont.

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT

34

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
1 5 10 15

Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
20 25 30

His Arg Gln Glu Ser Gly Phe Arg
35 40

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs

C3
cont.

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTTCCTGTG ATGTGCACCA

20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
1 5 10 15
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCAGARGARA CNATHAA

17

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

C3
cont.

Glu Glu Thr Ile Lys
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys
1 5

C3
ent.
(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCNGTGTTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

C3
cont.

- (A) LENGTH: 219 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT 60
GTGGTGGAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG 120
TGTCAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG 180
ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC 219

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC 18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATTT AGGTGACAC 19

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTCGCT GCAGC 25

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

C3
cont.

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1140 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 37..1086

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
 (B) LOCATION: 136..1086

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAACTC ATG ACT GTA CTC TAC CCA
 Met Thr Val Leu Tyr Pro
 -33 -30

54

GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA
 Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln
 -25 -20 -15

102

CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA
 His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile
 -10 -5 1 5

150

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| AAA | TTT | GCT | GCA | GCA | CAT | TAT | AAT | ACA | GAG | ATC | TTG | AAA | AGT | ATT | GAT | 198 |
| Lys | Phe | Ala | Ala | Ala | His | Tyr | Asn | Thr | Glu | Ile | Leu | Lys | Ser | Ile | Asp | |
| | | | | 10 | | | | | 15 | | | | | 20 | | |
| AAT | GAG | TGG | AGA | AAG | ACT | CAA | TGC | ATG | CCA | CGG | GAG | GTG | TGT | ATA | GAT | 246 |
| Asn | Glu | Trp | Arg | Lys | Thr | Gln | Cys | Met | Pro | Arg | Glu | Val | Cys | Ile | Asp | |
| | | | 25 | | | | | 30 | | | | | 35 | | | |
| GTG | GGG | AAG | GAG | TTT | GGA | GTC | GCG | ACA | AAC | ACC | TTC | TTT | AAA | CCT | CCA | 294 |
| Val | Gly | | Glu | Phe | Gly | Val | | Ala | Asn | Thr | Phe | | Lys | Pro | Pro | |
| | | 40 | | | | | 45 | | | | | 50 | | | | |
| TGT | GTG | TCC | GTC | TAC | AGA | TGT | GGG | GGT | TGC | TGC | AAT | AGT | GAG | GGG | CTG | 342 |
| Cys | Val | Ser | Val | Tyr | Arg | | Gly | Gly | Cys | Cys | Asn | Ser | Glu | Gly | Leu | |
| | | 55 | | | | 60 | | | | | 65 | | | | | |
| CAG | TGC | ATG | AAC | ACC | AGC | ACG | AGC | TAC | CTC | AGC | AAG | ACG | TTA | TTT | GAA | 390 |
| Gln | Cys | Met | Asn | Thr | Ser | Thr | Ser | Tyr | Leu | Ser | Lys | Thr | Leu | Phe | Glu | |
| | | 70 | | | 75 | | | | | 80 | | | | | 85 | |
| ATT | ACA | GTG | CCT | CTC | TCT | CAA | GGC | CCC | AAA | CCA | GTA | ACA | ATC | AGT | TTT | 438 |
| Ile | Thr | Val | Pro | Leu | Ser | Gln | Gly | Pro | Lys | Pro | Val | Thr | Ile | Ser | Phe | |
| | | | 90 | | | | | | 95 | | | | | 100 | | |
| GCC | AAT | CAC | ACT | TCC | TGC | CGA | TGC | ATG | TCT | AAA | CTG | GAT | GTT | TAC | AGA | 486 |
| Ala | Asn | His | | Ser | Cys | Arg | Cys | Met | Ser | Lys | Leu | Asp | Val | Tyr | Arg | |
| | | | 105 | | | | | 110 | | | | | 115 | | | |
| CAA | GTT | CAT | TCC | ATT | ATT | AGA | CGT | TCC | CTG | CCA | GCA | ACA | CTA | CCA | CAG | 534 |
| Gln | Val | His | Ser | Ile | Ile | Arg | Arg | Ser | Leu | Pro | Ala | Thr | Leu | Pro | Gln | |
| | | | 120 | | | | 125 | | | | | 130 | | | | |
| TGT | CAG | GCA | GCG | AAC | AAG | ACC | TGC | CCC | ACC | AAT | TAC | ATG | TGG | AAT | AAT | 582 |
| Cys | Gln | Ala | Ala | Asn | Lys | Thr | Cys | Pro | Thr | Asn | Tyr | Met | Trp | Asn | Asn | |
| | | 135 | | | | 140 | | | | | 145 | | | | | |
| CAC | ATC | TGC | AGA | TGC | CTG | GCT | CAG | GAA | GAT | TTT | ATG | TTT | TCC | TCG | GAT | 630 |
| His | Ile | Cys | Arg | Cys | Leu | Ala | Gln | Glu | Asp | Phe | Met | Phe | Ser | Ser | Asp | |
| | | 150 | | | 155 | | | | | 160 | | | | | 165 | |
| GCT | GGA | GAT | GAC | TCA | ACA | GAT | GGA | TTC | CAT | GAC | ATC | TGT | GGA | CCA | AAC | 678 |
| Ala | Gly | Asp | Asp | Ser | Thr | Asp | Gly | Phe | | His | Asp | Ile | Cys | Gly | Pro | |
| | | | | 170 | | | | 175 | | | | | | 180 | | |
| AAG | GAG | CTG | GAT | GAA | GAG | ACC | TGT | CAG | TGT | GTC | TGC | AGA | GCG | GGG | CTT | 726 |
| Lys | Glu | Leu | Asp | Glu | Glu | Thr | Cys | Gln | Cys | Val | Cys | Arg | Ala | Gly | Leu | |
| | | | 185 | | | | | 190 | | | | | 195 | | | |
| CGG | CCT | GCC | AGC | TGT | GGA | CCC | CAC | AAA | GAA | CTA | GAC | AGA | AAC | TCA | TGC | 774 |
| Arg | Pro | Ala | Ser | Cys | Gly | Pro | His | Lys | Glu | Leu | Asp | Arg | Asn | Ser | Cys | |
| | | 200 | | | | | 205 | | | | | 210 | | | | |
| CAG | TGT | GTC | TGT | AAA | AAC | AAA | CTC | TTC | CCC | AGC | CAA | TGT | GGG | GCC | AAC | 822 |
| Gln | Cys | Val | Cys | Lys | Asn | Lys | Leu | Phe | Pro | Ser | Gln | Cys | Gly | Ala | Asn | |
| | | 215 | | | | 220 | | | | | 225 | | | | | |
| CGA | GAA | TTT | GAT | GAA | AAC | ACA | TGC | CAG | TGT | GTA | TGT | AAA | AGA | ACC | TGC | 870 |
| Arg | Glu | Phe | Asp | Glu | Asn | Thr | Cys | Gln | Cys | Val | Cys | Lys | Arg | Thr | Cys | |
| | | 230 | | | 235 | | | | | 240 | | | | | 245 | |
| CCC | AGA | AAT | CAA | CCC | CTA | AAT | CCT | GGA | AAA | TGT | GCC | TGT | GAA | TGT | ACA | 918 |
| Pro | Arg | Asn | Gln | Pro | Leu | Asn | Pro | Gly | Lys | Cys | Ala | Cys | Glu | Cys | Thr | |
| | | | | 250 | | | | 255 | | | | | | 260 | | |
| GAA | AGT | CCA | CAG | AAA | TGC | TTG | TTA | AAA | GGA | AAG | AAG | TTC | CAC | CAC | CAA | 966 |
| Glu | Ser | Pro | Gln | Lys | Cys | Leu | Leu | Gly | Lys | Lys | Lys | Phe | His | His | Gln | |
| | | | 265 | | | | | 270 | | | | | 275 | | | |

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|---|------|
| ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT | 1014 |
| Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys | |
| 280 285 290 | |
| GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA | 1062 |
| Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser | |
| 295 300 305 | |
| TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT | 1116 |
| Tyr Trp Lys Arg Pro Gln Met Ser | |
| 310 315 | |
| TTTCTATTAT GGAAACTGT GTTG | 1140 |

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu
 -33 -30 -25 -20
 Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser
 -15 -10 -5
 Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu
 1 5 10 15
 Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro
 20 25 30
 Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn
 35 40 45
 Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
 50 55 60
 Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
 65 70 75
 Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
 80 85 90 95
 Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
 100 105 110
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
 115 120 125
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
 130 135 140
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
 145 150 155
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
 160 165 170 175
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
 180 185 190

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-50-

Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
195 200 205
Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
210 215 220
Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
225 230 235
Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
240 245 250 255
Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
260 265 270
Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
275 280 285
Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
290 295 300
Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
305 310 315

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGAGTGATTGTAGCTGCTGTG

22

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TATTGCAGCAACCCCCACATCT

22

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 196 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Met Arg Thr Trp Ala Cys Leu Leu Leu Leu Gly Cys Gly Tyr Leu Ala
1 5 10 15
His Ala Leu Ala Glu Glu Ala Glu Ile Pro Arg Glu Leu Ile Glu Arg
20 25 30
Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
35 40 45
Glu Ile Asp Ser Val Gly Ala Glu Asp Ala Leu Glu Thr Ser Leu Arg
50 55 60
Ala His Gly Ser His Ala Ile Asn His Val Pro Glu Lys Arg Pro Val
65 70 75 80
Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Ile Pro Ala Val Cys
85 90 95
Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
100 105 110
Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
115 120 125
Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
130 135 140
Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
145 150 155 160
Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
165 170 175
Cys Ala Cys Ala Thr Ser Asn Leu Asn Pro Asp His Arg Glu Glu Glu
180 185 190
Thr Asp Val Arg
195

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg
1 5 10 15
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met
20 25 30
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu
35 40 45
His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met
50 55 60

Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
65 70 75 80
Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
85 90 95
Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp
100 105 110
Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
115 120 125
Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
130 135 140
Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
145 150 155 160
Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
165 170 175
Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
180 185 190
Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
195 200 205
Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
210 215 220
Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
225 230 235 240
Ala

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cont.

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 149 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1 5 10 15
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
20 25 30
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35 40 45
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50 55 60
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65 70 75 80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Cys Gly Asp
130 135 140
Ala Val Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
1 5 10 15
Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
20 25 30
Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
35 40 45
Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
50 55 60
Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
65 70 75 80
Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro
85 90 95
Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
100 105 110
Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
115 120 125
Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
130 135 140
Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys Gln Arg Pro Thr Asp Cys
145 150 155 160
His Leu Cys Gly Asp Ala Val Pro Arg Arg
165 170

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Cys Asp Lys
130 135 140
Pro Arg Arg
145

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 191 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60

Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
130 135 140
Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln Thr
145 150 155 160
Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln
165 170 175
Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
180 185 190

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 215 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140

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Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145 150 155 160
Lys Ser Trp Ser Val Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys His
165 170 175
Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr
180 185 190
Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys
195 200 205
Arg Cys Asp Lys Pro Arg Arg
210 215

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
1 5 10 15
Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
20 25 30
Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
35 40 45
Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
50 55 60
Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
65 70 75 80
Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
85 90 95
Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
100 105 110
Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
115 120 125
Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Lys Lys Ser Val
130 135 140
Arg Gly Lys Gly Lys Gly Gln Lys Arg Lys Arg Lys Lys Ser Arg Tyr
145 150 155 160
Lys Ser Trp Ser Val Tyr Val Gly Ala Arg Cys Cys Leu Met Pro Trp
165 170 175
Ser Leu Pro Gly Pro His Pro Cys Gly Pro Cys Ser Glu Arg Arg Lys
180 185 190

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His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn
 195 200 205
 Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr
 210 215 220
 Cys Arg Cys Asp Lys Pro Arg Arg
 225 230

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1997 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCCGCCCCGC CTCACAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCCTCGCC 60
 CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC 120
 TTTTACCTGA CACCCGCGGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG 180
 GGAACGCGGA GCCCCGGACC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG 240
 GAGGAGCCCC GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCCGCCCC 300
 CCACCCCTGC CCCC GCCAGC GGACCGGTCC CCCACCCCG GTCTTCCAC C ATG CAC 357
 Met His
 1
 TIG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG 405
 Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Ala Leu
 5 10 15
 CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC 453
 Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser
 20 25 30
 GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT 501
 Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
 35 40 45 50
 TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA 549
 Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Ser Val
 55 60 65
 GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG 597
 Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
 70 75 80
 TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC 645
 Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
 85 90 95

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| CTC | AAC | TCA | AGG | ACA | GAA | GAG | ACT | ATA | AAA | TTT | GCT | GCA | GCA | CAT | TAT | 693 |
| Leu | Asn | Ser | Arg | Thr | Glu | Glu | Thr | Ile | Lys | Phe | Ala | Ala | Ala | His | Tyr | |
| 100 | | | | | | 105 | | | | | 110 | | | | | |
| AAT | ACA | GAG | ATC | TTG | AAA | AGT | ATT | GAT | AAT | GAG | TGG | AGA | AAG | ACT | CAA | 741 |
| Asn | Thr | Glu | Ile | Leu | Lys | Ser | Ile | Asp | Asn | Glu | Trp | Arg | Lys | Thr | Gln | |
| 115 | | | | | 120 | | | | | 125 | | | | | 130 | |
| TGC | ATG | CCA | CGG | GAG | GTG | TGT | ATA | GAT | GTG | GGG | AAG | GAG | TTT | GGA | GTC | 789 |
| Cys | Met | Pro | Arg | Glu | Val | Cys | Ile | Asp | Val | Gly | Lys | Glu | Phe | Gly | Val | |
| | | | | 135 | | | | | 140 | | | | | 145 | | |
| GCG | ACA | AAC | ACC | TTC | TTT | AAA | CCT | CCA | TGT | GTG | TCC | GTC | TAC | AGA | TGT | 837 |
| Ala | Thr | Asn | Thr | Phe | Phe | Lys | Pro | Pro | Cys | Val | Ser | Val | Tyr | Arg | Cys | |
| | | | 150 | | | | 155 | | | | | | 160 | | | |
| GGG | GGT | TGC | TGC | AAT | AGT | GAG | GGG | CTG | CAG | TGC | ATG | AAC | ACC | AGC | ACG | 885 |
| Gly | Gly | Cys | Cys | Asn | Ser | Glu | Gly | Leu | Gln | Cys | Met | Asn | Thr | Ser | Thr | |
| | | 165 | | | | | 170 | | | | | 175 | | | | |
| AGC | TAC | CTC | AGC | AAG | ACG | TTA | TTT | GAA | ATT | ACA | GTG | CCT | CTC | TCT | CAA | 933 |
| Ser | Tyr | Leu | Ser | Lys | Thr | Leu | Phe | Glu | Ile | Thr | Val | Pro | Leu | Ser | Gln | |
| | 180 | | | | | 185 | | | | | 190 | | | | | |
| GGC | CCC | AAA | CCA | GTA | ACA | ATC | AGT | TTT | GCC | AAT | CAC | ACT | TCC | TGC | CGA | 981 |
| Gly | Pro | Lys | Pro | Val | Thr | Ile | Ser | Phe | Ala | Asn | His | Thr | Ser | Cys | Arg | |
| 195 | | | | | 200 | | | | | 205 | | | | | 210 | |
| TGC | ATG | TCT | AAA | CTG | GAT | GTT | TAC | AGA | CAA | GTT | CAT | TCC | ATT | ATT | AGA | 1029 |
| Cys | Met | Ser | Lys | Leu | Asp | Val | Tyr | Arg | Gln | Val | His | Ser | Ile | Ile | Arg | |
| | | | | 215 | | | | | 220 | | | | | 225 | | |
| CGT | TCC | CTG | CCA | GCA | ACA | CTA | CCA | CAG | TGT | CAG | GCA | GCG | AAC | AAG | ACC | 1077 |
| Arg | Ser | Leu | Pro | Ala | Thr | Leu | Pro | Gln | Cys | Gln | Ala | Ala | Lys | Thr | | |
| | | | 230 | | | | | 235 | | | | | 240 | | | |
| TGC | CCC | ACC | AAT | TAC | ATG | TGG | AAT | AAT | CAC | ATC | TGC | AGA | TGC | CTG | GCT | 1125 |
| Cys | Pro | Thr | Asn | Tyr | Met | Trp | Asn | Asn | His | Ile | Cys | Arg | Cys | Leu | Ala | |
| | | 245 | | | | | 250 | | | | | 255 | | | | |
| CAG | GAA | GAT | TTT | ATG | TTT | TCC | TCG | GAT | GCT | GGA | GAT | GAC | TCA | ACA | GAT | 1173 |
| Gln | Glu | Asp | Phe | Met | Phe | Ser | Ser | Asp | Ala | Gly | Asp | Asp | Ser | Thr | Asp | |
| | 260 | | | | | 265 | | | | | 270 | | | | | |
| GGA | TTC | CAT | GAC | ATC | TGT | GGA | CCA | AAC | AAG | GAG | CTG | GAT | GAA | GAG | ACC | 1221 |
| Gly | Phe | His | Asp | Ile | Cys | Gly | Pro | Asn | Lys | Glu | Leu | Asp | Glu | Glu | Thr | |
| 275 | | | | | 280 | | | | | 285 | | | | | 290 | |
| TGT | CAG | TGT | GTC | TGC | AGA | GCG | GGG | CTT | CGG | CCT | GCC | AGC | TGT | GGA | CCC | 1269 |
| Cys | Gln | Cys | Val | Cys | Arg | Ala | Gly | Leu | Arg | Pro | Ala | Ser | Cys | Gly | Pro | |
| | | | | 295 | | | | | 300 | | | | | 305 | | |
| CAC | AAA | GAA | CTA | GAC | AGA | AAC | TCA | TGC | CAG | TGT | GTC | TGT | AAA | AAC | AAA | 1317 |
| His | Lys | Glu | Leu | Asp | Arg | Asn | Ser | Cys | Gln | Cys | Val | Cys | Lys | Asn | Lys | |
| | | | 310 | | | | 315 | | | | | | 320 | | | |
| CTC | TTC | CCC | AGC | CAA | TGT | GGG | GCC | AAC | CGA | GAA | TTT | GAT | GAA | AAC | ACA | 1365 |
| Leu | Phe | Pro | Ser | Gln | Cys | Gly | Ala | Asn | Arg | Glu | Phe | Asp | Glu | Asn | Thr | |
| | | 325 | | | | 330 | | | | | | 335 | | | | |
| TGC | CAG | TGT | GTA | TGT | AAA | AGA | ACC | TGC | CCC | AGA | AAT | CAA | CCC | CTA | AAT | 1413 |
| Cys | Gln | Cys | Val | Cys | Lys | Arg | Thr | Cys | Pro | Arg | Asn | Gln | Pro | Leu | Asn | |
| | 340 | | | | | 345 | | | | | 350 | | | | | |
| CCT | GGA | AAA | TGT | GCC | TGT | GAA | TGT | ACA | GAA | AGT | CCA | CAG | AAA | TGC | TTG | 1461 |
| Pro | Gly | Lys | Cys | Ala | Cys | Glu | Cys | Thr | Glu | Ser | Pro | Gln | Lys | Cys | Leu | |
| 355 | | | | | 360 | | | | | 365 | | | | | 370 | |

C3
cont.



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1801 |
| Filed: January 12, 1996 |) | Examiner: Lathrop, B. |

**AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents
Washington, D.C. 20231*

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;
(B) Exhibits 1, 2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and 1.825(b);
4. Declaration under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on November 26, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

1. **Small Entity Status**

- ☒ Small entity status has been established and is still effective.

2. **Extension of Time**

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

| EXTENSION (Months) | FEE FOR LARGE ENTITY | | FEE FOR SMALL ENTITY | |
|-----------------------|----------------------|------------|----------------------|----------|
| One Month | | \$110.00 | | \$55.00 |
| Two Months | | \$400.00 | | \$200.00 |
| Three Months | | \$950.00 | X | \$475.00 |
| Four Months | | \$1,510.00 | | \$755.00 |

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$475.00

- ☐ An extension for _____ month(s) has already been secured and the fee paid therefor of \$_____ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$0

Extension Fee Due With This Request \$475.00

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

| | | | | | SMALL ENTITY | | OTHER THAN A SMALL ENTITY | |
|--|----------------------------------|---------------------------------|----|---------------|--------------|----------------|---------------------------|----------------|
| | Claims Remaining After Amendment | Highest No. Previously Paid For | | Present Extra | Rate | Additional Fee | Rate | Additional Fee |
| TOTAL | 33 | MINUS | 20 | 13 | X11 = | \$143 | X22 = | \$ |
| INDEP. | 5 | MINUS | 3 | 2 | X41 = | \$82 | X82 = | \$ |
| = First Presentation of Multiple Dependent Claim | | | | | + 135 = | \$135 | + 270 = | \$ |
| TOTAL ADDITIONAL FEE | | | | | | \$360 | OR | \$ |

4. **Method of Payment of Fees**

Attached are checks in the amount of \$475 and \$360.

- ☐ Charge Deposit Account No. 13-2855
in the amount of: \$ _____
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By: 

David A. Gass
Reg. No: 38,153

November 26, 1997



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|------------------------|
| Applicant(s): |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No: 08/585,895 |) | Group Art Unit: 1801 |
| Filed: January 12, 1996 |) | Examiner: Lathrop, B. |

AMENDMENT TRANSMITTAL WITH
PETITION FOR EXTENSION OF TIME

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents for the above application:

1. Amendment and Reply Pursuant to 37 C.F.R. §§ 1.111 and 1.115, including:
(A) new pages 40-60 comprising a paper copy of a substitute Sequence Listing;
(B) Exhibits 1, 2 and 3;
2. Computer-readable copy of substitute Sequence Listing;
3. Statement Pursuant to 37 C.F.R. 1.825(a) and 1.825(b);
4. Declaration under 37 C.F.R. § 1.132 of Dr. Kari Alitalo;
5. Declaration of Biological Culture Deposit in Compliance with Budapest Treaty Requirements;
6. Check in the amount of \$475.00 in payment of fee for extension of time; and
7. Check in the amount of \$360 in payment of fee for extra claims.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on November 26, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.


David A. Gass

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed as Application Serial No. 08/585,895. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) originating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:



| (Application Serial Number) | (Country) | (Day/Month/Year Filed) | Priority Claimed |
|-----------------------------|-----------|------------------------|---|
| 950634 | Finland | 13 February 1995 | <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No |

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

| (Application Serial Number) | (Day/Month/Year Filed) |
|-----------------------------|------------------------|
|-----------------------------|------------------------|

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurs between the filing date of the prior application(s) and the national or PCT international filing date of this application:

| (Application Serial Number) | (Day/Month/Year Filed) | (Status-Patented, Pending or Abandoned) |
|-----------------------------|------------------------|---|
| 08/340,011 | 14 November 1994 | Pending |
| 08/510,133 | 01 August 1995 | Pending |

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

| | | | |
|----------------------------|-------------------------------|------------------------------|---------------------------------|
| Alvin D. Shulman (19,412) | Trevor B. Jinks (23,542) | Richard A. Schauer (30,890) | James J. Napoli (22,361) |
| Donald J. Bratt (19,490) | Timothy J. Vachon (26,348) | Anthony Munno (30,920) | Richard M. La Borge (32,254) |
| Jean J. Murray (22,111) | Carl E. Moore, Jr. (26,487) | Christine A. Dutzik (31,245) | Jeffrey W. Smith (33,455) |
| Allen H. Germain (22,218) | Richard H. Anderson (28,526) | Kevin D. Hogg (31,839) | Douglas C. Hochstetler (33,710) |
| Nate F. Scarpelli (22,320) | Patrick D. Estel (28,877) | Jeffrey S. Sharp (31,879) | Cynthia L. Schaller (34,245) |
| Edward M. O'Toole (22,477) | James P. Zeller (28,491) | Donald I. Pochopkin (32,167) | Robert M. Germain (34,824) |
| Michael F. Baron (23,447) | William E. McCracken (30,195) | Martin J. Hirsch (32,237) | David A. Goss (38,153) |

Send correspondence to: David A. Goss

| FIRM NAME | PHONE NO | STREET | CITY & STATE | ZIP CODE |
|---|--------------|--|-------------------|------------|
| Marshall, O'Toole, Germain, Murray & Baron | 312 474 6300 | 6300 South Tower 233 South Wacker Drive | Chicago, Illinois | 60606-6402 |

| | |
|-------------------------------------|------------------------------|
| Full Name of First or Sole Inventor | Citizenship |
| Kari Alitalo | Finland |
| Residence Address - Street | Post Office Address - Street |
| Nyyrikintie 4A | Same |
| City (Zip) | City (Zip) |
| 02100 Espoo | Same |
| State or Country | State or Country |
| FINLAND | Same |
| Date | Signature |
| Dec 6, 1996 | [Signature] |

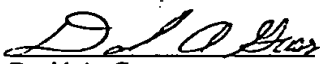
See second page for additional inventor

See reverse for retrieval rules & statute

| | |
|-------------------------------|------------------------------|
| Second Name (surname, if any) | Citizenship |
| Vladimir Joukov | Russia |
| Residence Address - Street | Post Office Address - Street |
| Topeliuksenkatu 32G8 | Same |
| City (Zip) | City (Zip) |
| 00290 Helsinki | Same |
| State or Country | State or Country |
| FINLAND | Same |
| Date | Signature |
| ■ Aug. 6, 1946 | ■ V. Joukov |

PATENT
28967/33072

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|---|
| In re Application of: |) | I hereby certify that this paper is being |
| Alitalo et al. |) | deposited with the United States Postal |
| Serial No.: 08/585,895 |) | Service as first class mail, postage |
| Filed: January 12, 1996 |) | prepaid, in an envelope addressed to: |
| Title: RECEPTOR LIGAND |) | Assistant Commissioner for Patents |
| Art Unit: 1801 |) | Washington, D.C. 20231, on this date: |
| Examiner: Lathrop, B. |) | Dated: <u>Nov. 26, 1997</u> |
| |) |  |
| |) | David A. Gass |
| |) | Registration No. 38,153 |

DECLARATION OF BIOLOGICAL CULTURE DEPOSIT
IN COMPLIANCE WITH BUDAPEST TREATY REQUIREMENTS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, the undersigned, declare that:

1. I am an inventor of the subject matter of the above-identified patent application.
2. The plasmid designated FLT4-L, described in the specification of the above-identified application at pages 28-29 (and elsewhere), was deposited on 24 July 1995 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852, under the terms of the Budapest Treaty. This plasmid was assigned ATCC accession number 97231. A copy of the ATCC deposit receipt, confirming viability of the deposit, is attached hereto.

3. With respect to the permanence of the deposit, the ATCC is an official depository in accordance with the Budapest Treaty for the above-deposited material, and I affirm that, should the plasmid identified in paragraph 2 mutate, become non-viable, or be inadvertently destroyed, I will replace it for at least thirty (30) years from the date of the original deposit, or for at least five (5) years from the date of the most recent request for release of a sample, or for the enforceable life of any patent issued on the above-mentioned application, whichever period is longest.

4. With respect to availability of the plasmid identified in paragraph 2, I affirm that the deposit has been made under conditions of assurance of (a) ready accessibility thereto by the public if an enforceable patent is granted whereby all restrictions to the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent [MPEP §608.01 (p)], and (b) access to the deposit will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 C.F.R. §1.14 and 35 U.S.C. §122.

5. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Kari Alitalo
Kari Alitalo



American Type Culture Collection

12301 Parklawn Drive • Rockville, MD 20852 USA • Telephone: (301) 231-5528 Telex: 898-055 ATCCNORTH • FAX: 301-770-2587

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

University of Helsinki
Attention: Kari Alitalo
Molecular/Cancer Biology Laboratory
P.O. Box 21 (Haartmaninkatu 3)
SF-00014, HELSINKI, FINLAND

Deposited on Behalf of: Kari Alitalo and Vladimir Joukov

Identification Reference by Depositor:

ATCC Designation

Plasmid, FLT4-L

97231

The deposit was accompanied by: ☐ a scientific description ☐ a proposed taxonomic description indicated above.

The deposit was received July 24, 1995 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:

☒ We will not inform you of requests for the strain.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

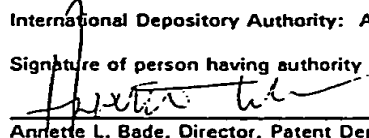
If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years after the date of deposit, and for a period of at least five years after the most recent request for a sample. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested August 1, 1995. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:


Annette L. Bade, Director, Patent Depository

Date: August 9, 1995

cc: Thomas C. Meyers



PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|--------------------------------------|
| In re Application of: |) | I hereby certify that this paper is |
| |) | being deposited with the United |
| Alitalo et al. |) | States Postal Service as first class |
| |) | mail, postage prepaid, in an |
| Serial No. 08/585,895 |) | envelope addressed to: Assistant |
| |) | Commissioner for Patents, |
| Filed: January 12, 1996 |) | Washington, D.C. 20231, on this |
| |) | date: |
| For: RECEPTOR LIGAND |) | Dated: <u>November 26, 1997</u> |
| |) | |
| Art Unit: 1801 |) | <u>David A. Gass</u> |
| |) | David A. Gass |
| Examiner: Lathrop, B. |) | |

STATEMENT PURSUANT TO 37 C.F.R. §1.825(a) and §1.825(b)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby state that the content of the paper and computer-readable forms of the substitute Sequence Listing submitted herewith, for entry as part of the above-identified application, are the same as each other and do not introduce new matter into the disclosure of the application. All of the amendments embodied in the substitute Sequence Listing filed herewith find support in the application as originally filed.

SEQ ID NOs: 1-31 and 34-35 of the original and substitute Sequence Listings are identical. Therefore, no new matter has been introduced in these sequences.

SEQ ID NOs: 36-43 have been added to the substitute Sequence Listing pursuant to instructions from the Patent Office to include sequences therein that are depicted in Figure 10 of the application. Because these eight sequences all find support in Figure 10 as originally filed, they do not introduce

new matter. Appropriate cross-references to SEQ ID NOs: 36-43 have been included in the brief description of the drawing.

SEQ ID NOs: 32-33 of the original and substitute Sequence Listings are identical. However, the amino acid numbering of these sequences has been amended in the substitute sequence listing by identifying the 34th residue in the substitute sequence listing as residue 1. (In the original sequence listing, the 33rd residue was identified as residue 1.) This amendment finds support throughout the specification as originally filed. For example, the description of the amino terminus of a mature form of VEGF-C is found in the specification at p. 23, lines 5-10, and is confirmed at page 25, line 27, to page 26, line 6 (from which it is apparent that the first 46 codons comprise 33 "signal sequence" residues and 13 amino acid residues of a secreted Flt4 ligand). From these excerpts of the specification that identify the amino terminus of a mature VEGF-C protein, it is clear that the residues of SEQ ID NO: 33 as originally filed were misnumbered by one residue. Because the error and its proper correction are apparent from the specification as originally filed, the corrections to SEQ ID NOs: 32-33 do not introduce new matter.

SEQ ID NOs: 44-45 of the substitute Sequence Listing depict a 1997 base pair nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the ATCC and cross-referenced at pp. 28-29 of the patent application as filed. These sequences are inherent properties of the deposited plasmid and thus find support in the deposited plasmid itself. See *Kennecott Corp. v. Kyocera International Inc.* 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date); *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985); see also Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo (filed herewith) at ¶¶ 2-5.


In accordance with 37 C.F.R. §1.68, I hereby declare that the foregoing statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, IL 60606-6402
Telephone: (312) 474-6300

November 26, 1997


David A. Gass
Registration No. 38,153



PATENT
28967/33072

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No.: 08/585,895

Filed: January 12, 1996


Title: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is being
) deposited with the United States Postal
) Service as first class mail, postage
) prepaid, in an envelope addressed to:
) Assistant Commissioner for Patents
) Washington, D.C. 20231, on this date:

) Dated: Nov. 26, 1997

) 
) David A. Gass
) Registration No. 38,153

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated May 28, 1997, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

Isolation of VEGF-C protein and cDNA

2. The present invention relates generally to a protein ligand for Flt4 receptor tyrosine kinase (VEGFR-3), which our research team has designated "VEGF-C." As taught in Example 14 of the patent application, VEGF-C also stimulates KDR/Flk-1 receptor tyrosine kinase (VEGFR-2). Our research team purified a VEGF-C protein that we discovered in conditioned media from a PC-3 prostatic adenocarcinoma cell line. We demonstrated that this protein bound to the extracellular domain of Flt4 and stimulated Flt4 phosphorylation. (See the patent application at Examples 4-5, for example.) Using SDS polyacrylamide gel electrophoresis, the VEGF-C protein was originally determined to have a molecular weight of about 23 kilodaltons. This measurement is in good

agreement with subsequent measurements of VEGF-C that we have recombinantly expressed in multiple cell lines, where we have determined the molecular weight to be about 21-23 kD.)

3. We sequenced the amino terminus of this purified VEGF-C protein as taught in the patent application in Example 5. (See especially p. 23.) I hereby reaffirm that our sequencing data from this protein is correctly reported in the patent application at p. 23 and in SEQ ID NO: 13.

4. As taught in Examples 6-10 of the patent application, we used the amino terminal amino acid sequence taught in the patent application to obtain a cDNA encoding VEGF-C. A plasmid containing the cDNA that is described in Example 11 of the patent application was deposited with the American Type Culture Collection and accorded ATCC accession number 97231.

5. The patent application describes a partial nucleotide sequence and a 350 amino acid open reading frame of the deposited VEGF-C cDNA. (See SEQ ID NOs: 32 and 33 of the patent application.) In the amendment filed herewith, these sequences have been amended such that the designation of residue "1" therein corresponds with the first residue of VEGF-C purified from PC-3 conditioned medium as described in the patent application. (See also paragraph 3, above.) Amended SEQ ID NOs: 32-33 are attached hereto as Exhibit A. Complete sequencing of the cDNA subsequently demonstrated that the translated open reading frame is actually 419 amino acids: it extends 69 codons upstream of what is reported in SEQ ID NO: 33. Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45. I shall use the term "prepro-VEGF-C" herein to refer to a polypeptide consisting of this 419 amino acid sequence.

6. As taught in the patent application (e.g., at p. 11), the carboxyl-terminal amino acid sequences encoded by the VEGF-C cDNA show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence that was

known in the art. (See Dignam and Case, *Gene*, 88:133-40 (1990); and Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990), both of record and cited in the patent application). The distinctive BR3P cysteine motifs (Cys-Xaa_n-Cys-Xaa-Cys-Xaa-Cys, wherein Xaa is any residue and n is variable) occur at least four times in the carboxy-terminal portion of VEGF-C (see Cys residues in Exhibit B at positions 280, 291, 293, and 295; positions 304, 315, 317, and 319; positions 328, 339, 341, and 343; and positions 347, 358, 360, and 362).

**VEGF-C processing and determination of
VEGF-C fragments that bind to Flt4.**

7. The Patent application teaches that the protein encoded by the VEGF-C gene is proteolytically processed, and teaches procedures to characterize this processing, such as analysis using antibodies and pulse-chase experiments. The application further teaches to screen truncated forms of VEGF-C (e.g., deletion fragments) to determine the portions of VEGF-C that are necessary to bind and stimulate Flt4. (See, e.g., pp. 29-30 of the patent application.) Using techniques such as those described at pp. 29-30 of the patent application and mutational analysis, our research team has extensively characterized the processing of human prepro-VEGF-C in mammalian cell lines.

A. Our results from pulse-chase experiments indicate that the apparent first proteolytic processing of human prepro-VEGF-C involves cleavage of a signal peptide of about 31 residues, leaving residues 32-419 (hereinafter "pro-VEGF-C"). Pro-VEGF-C has an apparent molecular weight of about 55-58 kD.

B. We next observed that pro-VEGF-C is cleaved, either intracellularly or at the cell surface, into polypeptides of about 29 kD and about 31-32 kD (when assessed by SDS-PAGE under reducing conditions). The ~32 kD polypeptide binds the extracellular domain of Flt4 receptor tyrosine kinase with high affinity. (See Example 13 of the patent application.) The ~32 kD polypeptide was purified with immunoaffinity chromatography using an anti-VEGF-C antibody. The amino-terminus of

this purified polypeptide was determined to correspond to position 32 of the sequence shown in Exhibit B. Thus, the ~32 kD polypeptide represents the amino-terminal product of this proteolytic cleavage. Sequencing of the ~29 kD polypeptide indicated that cleavage occurred after amino acid 227 of the 419 amino acid sequence depicted in Exhibit B. (Amino acid 227 corresponds to residue 125 of SEQ ID NO: 33 in the patent application (Exhibit A).) This carboxy-terminal fragment of about 29 kD presumably includes residues 228-419 of the sequence depicted in Exhibit B (residues 126-317 of SEQ ID NO: 33). Thus, the ~29 kD polypeptide includes all of the Balbiani ring 3 protein cysteine motifs of VEGF-C (see paragraph 6 above). These results indicate that polypeptide fragments of the sequences depicted in Exhibits A or B that lack any domain having cysteine motifs of a Balbiani ring 3 protein (e.g., that lack the ~29 kD carboxy-terminal fragment) remain capable of binding with the extracellular domain of Flt4.

C. We also have observed forms of VEGF-C that reflect further proteolytic processing at the amino terminus. For the purpose of this declaration, I shall collectively refer to forms of VEGF described below as "mature VEGF-C."

- i. As indicated in paragraph 3, above, VEGF-C isolated from conditioned medium of PC-3 cells has an amino terminus corresponding to amino acid 103 in Exhibit B (i.e., amino acid 1 of SEQ ID NO: 33 (Exhibit A)).
- ii. We have sequenced VEGF-C that was recombinantly expressed in 293-EBNA cells (as described in Example 11 of the patent application) and determined that the amino terminus of this form corresponds with position 112 of the sequence shown in Exhibit B (i.e., position 10 of SEQ ID NO: 33 (Exhibit A)).

8. Our research team modified the human VEGF-C cDNA to recombinantly produce a fragment consisting of amino acids 104-213 of the 419 amino acid polypeptide in yeast (i.e., residues 2-111 of SEQ ID NO: 33). This fragment was shown to bind Flt4 and stimulate phosphorylation of both Flt4 (VEGFR-3) and KDR (VEGFR-2). In another experiment, a fragment lacking residues 1-112 of the 419 amino acid polypeptide retained receptor binding activity.

9. Collectively, the experimental results described in the preceding paragraphs indicate that polypeptides lacking amino acids 1-112 and 214-419 of the 419 residue amino acid sequence shown in Exhibit B retain Flt4 binding and stimulating activities. Stated differently, we have experimental evidence to indicate that a polypeptide corresponding to positions 11-112 of SEQ ID NO: 33 will retain Flt4 binding and stimulating activities. Moreover, one skilled in the art understands from the patent application how to perform receptor binding and phosphorylation assays, to localize further the portion of SEQ ID NO: 33 that is required for activity.

**The application enables one to obtain
VEGF-C-encoding cDNAs from non-human sources**

10. I infer from page 5 of the Office action that the Patent Office has rejected a claim of the application in part because of the lack of a claim limitation with respect to the source animal for VEGF-C. This section of the declaration provides evidence that the teachings in the patent application of a human VEGF-C cDNA, combined with the teachings that VEGF-C protein binds Flt4 (VEGFR-3) and VEGFR-2, enable one to obtain VEGF-C-encoding cDNAs from non-human sources.

11. To clone a murine VEGF-C cDNA, approximately 1×10^6 bacteriophage lambda clones of a commercially-available 12 day mouse embryonal cDNA library (lambda EXlox library, Novagen, catalog number 69632-1) were screened with a radiolabeled fragment of human VEGF-C cDNA containing nucleotides 495 to 1661 of the nucleotide sequence shown in Exhibit B. One positive clone was isolated.

12. A 1323 bp *EcoRI/HindIII* fragment of the insert of the isolated mouse cDNA clone was subcloned into the corresponding sites of the pBluescript SK+ vector (Stratagene) and sequenced. The cDNA sequence of this clone was homologous to the human VEGF-C sequence reported herein, except that about 710 bp of 5'-end sequence present in the human clone was not present in the mouse clone.

13. For further screening of mouse cDNA libraries, a *HindIII-BsrXI* (*HindIII* site is from the pBluescript SK+ polylinker) fragment of 881 bp from the coding region of the mouse cDNA clone was radiolabeled and used as a probe to screen two additional mouse cDNA libraries. Two additional cDNA clones from an adult mouse heart ZAP II cDNA library (Stratagene, catalog number 936306) were identified. Three additional clones also were isolated from a mouse heart 5'-stretch-plus cDNA library in λ gt11 (Clontech Laboratories, Inc., catalog number ML5002b). Of the latter three clones, one was found to contain an insert of about 1.9 kb. The insert of this cDNA clone was subcloned into *EcoRI* sites of pBluescript SK+ vector and both strands of this clone were completely sequenced, resulting in the nucleotide and deduced amino acid sequences shown in Exhibit C. It is expected that the mouse VEGF-C polypeptide depicted in Exhibit C is processed into a mature mouse VEGF-C protein, in a manner analogous to the processing of the human prepro-VEGF-C.

14. The foregoing results demonstrate the utility of human VEGF-C-encoding polynucleotides of the invention for identifying and isolating polynucleotides encoding other non-human mammalian VEGF-C proteins. Such identified and isolated polynucleotides, in turn, can be expressed (using procedures similar to those described in the patent application for human VEGF-C) to produce recombinant polypeptides corresponding to non-human mammalian forms of VEGF-C.

15. The identity of the mouse protein as VEGF-C was confirmed by recombinantly expressing the above-described mouse cDNA, and analyzing the expressed proteins.

A. The 1.8 kb mouse VEGF-C cDNA was cloned as an *EcoRI* fragment into the retroviral expression vector pBabe-puro containing the SV40 early promoter region [Morgenstern *et al.*, *Nucl. Acids Res.*, 18:3587-3595 (1990)], and transfected into the Bosc23 packaging cell line [Pearet *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 90:8392-8396 (1994)] by the calcium-phosphate precipitation method. For comparison, Bosc23 cells also were transfected with the previously-described human VEGF-C construct in the pREP7 expression vector. The expressed proteins were immunoprecipitated with polyclonal antibodies raised against mature human VEGF-C.

B. Immunoprecipitation of VEGF-C from media of transfected and metabolically-labelled cells revealed bands of approximately $30\text{-}32 \times 10^3$ M, (a doublet) and $22\text{-}23 \times 10^3$ M, in 12.5% SDS-PAGE. These bands were not detected in samples from nontransfected or mock-transfected cells. These results demonstrate that antibodies raised against human VEGF-C recognize the corresponding mouse protein.

C. For receptor binding experiments, 1 ml aliquots of media from metabolically-labelled Bosc23 cells were incubated with VEGFR-3 extracellular domain, covalently coupled to sepharose, for 4 hours at 4°C with gentle mixing. (See Examples 4 and 5 in the patent application.) The sepharose beads were washed four times with ice-cold phosphate buffered saline (PBS), and the samples were analyzed by gel electrophoresis as described in Joukov *et al.*, *EMBO J.*, 15:290-298 (1996).

D. Similar $30\text{-}32 \times 10^3$ M, doublet and $22\text{-}23 \times 10^3$ M, polypeptide bands were obtained in the receptor binding assay as compared to the immunoprecipitation assay. In additional experiments, mouse VEGF-C appeared to be a potent inducer of VEGFR-3 autophosphorylation, too. Thus, the putative mouse VEGF-C binds and stimulates human VEGFR-3, confirming its identity. The slightly faster mobility of the mouse VEGF-C polypeptides that was observed may be caused by the four amino acid

residue difference observed in sequence analysis (residues H88-E91).
Murine VEGF-C appeared to bind VEGFR-2 with lower affinity.

16. The human VEGF-C cDNA also was used to design probes for successfully isolating a quail VEGF-C cDNA from a quail cDNA library. A fragment of the human VEGF-C cDNA comprising nucleotides 495-1661 of Exhibit B was obtained by PCR amplification, cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions, and amplified. The insert was isolated by *Eco* RI digestion and preparative gel electrophoresis and then labelled using radioactive dCTP and random priming. A cDNA library made from quail embryos of stage E-4 in pcDNA-1 vector (Invitrogen) was then screened using this probe. About 200,000 colonies were plated and filter replicas were hybridized with the radioactive probe under reduced stringency conditions (washes at 42°C with a wash solution comprising 2x SSC/0.1% SDS). Nine positive clones were identified and secondarily plated. Two of the nine clones hybridized in secondary screening. The purified clones (clones 1 and 14) had approximately 2.7 kb *Eco* RI inserts. Both clones were amplified and then sequenced using the T7 and SP6 primers (annealing to the vector). In addition, an internal *Sph* I restriction endonuclease cleavage site was identified about 1.9 kb from the T7 primer side of the vector and used for subcloning 5'- and 3'- *Sph* I fragments, followed by sequencing from the *Sph* I end of the subclones. The sequences obtained were identical from both clones and showed a high degree of similarity to the human VEGF-C coding region. Subsequently, walking primers were made in both directions and double-stranded sequencing was completed for 1743 base pairs, including the full-length open reading frame.

17. The cDNA sequence obtained includes a long open reading frame and 5' untranslated region. The DNA and deduced amino acid sequences for the quail cDNA are set forth in Exhibit D. Studies performed with the putative quail VEGF-C cDNA have shown that its protein product is secreted from transfected cells and interacts with avian VEGFR-3 and VEGFR-2, further confirming the conclusion that the cDNA encodes a quail VEGF-C protein.

18. As shown in Exhibit E, the human, murine, and avian (quail) VEGF-C precursor amino acid sequences share a significant degree of conservation. This high degree of homology confirms the likelihood of success of attempts to isolate VEGF-C encoding sequences from other species, especially vertebrate species, and more particularly mammalian and avian species, using human VEGF-C-encoding polynucleotides taught in the patent application as probes and using standard molecular biological techniques. The identity of putative VEGF-C-encoding cDNAs is confirmed using receptor binding studies such as the studies described in the patent application.

Certification

19. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

November 20, 1997
Date

Jan Ombak
Kari Alitalo

EXHIBIT A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

| | | |
|---|-------------------------|----|
| GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAAC | ATG ACT GTA CTC TAC CCA | 54 |
| | Met Thr Val Leu Tyr Pro | |
| | -33 -30 | |
| GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA | 102 | |
| Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln | | |
| | -25 -20 -15 | |
| CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA | 150 | |
| His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile | | |
| | -10 -5 1 5 | |
| AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT | 198 | |
| Lys Phe Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp | | |
| | 10 15 20 | |
| AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT | 246 | |
| Asn Glu Trp Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp | | |
| | 25 30 35 | |
| GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA | 294 | |
| Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro | | |
| | 40 45 50 | |
| TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG | 342 | |
| Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu | | |
| | 55 60 65 | |
| CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA | 390 | |
| Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu | | |
| | 70 75 80 85 | |
| ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT | 438 | |
| Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe | | |
| | 90 95 100 | |
| GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA | 486 | |
| Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg | | |
| | 105 110 115 | |
| CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG | 534 | |
| Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln | | |
| | 120 125 130 | |
| TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT | 582 | |
| Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn | | |
| | 135 140 145 | |
| CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT | 630 | |
| His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp | | |
| | 150 155 160 165 | |
| GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC | 678 | |
| Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn | | |
| | 170 175 180 | |
| AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT | 726 | |
| Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu | | |
| | 185 190 195 | |
| CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC | 774 | |
| Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys | | |
| | 200 205 210 | |

| 200 | 205 | 210 | |
|---|-----|-----|------|
| CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn 215 220 225 | | | 822 |
| CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys 230 235 240 245 | | | 870 |
| CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr 250 255 260 | | | 918 |
| GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln 265 270 275 | | | 966 |
| ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys 280 285 290 | | | 1014 |
| GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser 295 300 305 | | | 1062 |
| TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTTCCTA GTTCATCGAT Tyr Trp Lys Arg Pro Gln Met Ser 310 315 | | | 1116 |
| TTTCTATTAT GGAAACTGT GTTG | | | 1140 |

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 350 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

| |
|--|
| Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu -33 -30 -25 -20 |
| Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser -15 -10 -5 |
| Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu 1 5 10 15 |
| Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro 20 25 30 |
| Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn 35 40 45 |
| Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys 50 55 60 |
| Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu 65 70 75 |
| Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys 80 85 90 95 |

| | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Val | Thr | Ile | Ser | Phe | Ala | Asn | His | Thr | Ser | Cys | Arg | Cys | Met | Ser | 100 | 105 | 110 |
| Lys | Leu | Asp | Val | Tyr | Arg | Gln | Val | His | Ser | Ile | Ile | Arg | Arg | Ser | Leu | 115 | 120 | 125 |
| Pro | Ala | Thr | Leu | Pro | Gln | Cys | Gln | Ala | Ala | Asn | Lys | Thr | Cys | Pro | Thr | 130 | 135 | 140 |
| Asn | Tyr | Met | Trp | Asn | Asn | His | Ile | Cys | Arg | Cys | Leu | Ala | Gln | Glu | Asp | 145 | 150 | 155 |
| Phe | Met | Phe | Ser | Ser | Asp | Ala | Gly | Asp | Asp | Ser | Thr | Asp | Gly | Phe | His | 160 | 165 | 170 |
| Asp | Ile | Cys | Gly | Pro | Asn | Lys | Glu | Leu | Asp | Glu | Glu | Thr | Cys | Gln | Cys | 180 | 185 | 190 |
| Val | Cys | Arg | Ala | Gly | Leu | Arg | Pro | Ala | Ser | Cys | Gly | Pro | His | Lys | Glu | 195 | 200 | 205 |
| Leu | Asp | Arg | Asn | Ser | Cys | Gln | Cys | Val | Cys | Lys | Asn | Lys | Leu | Phe | Pro | 210 | 215 | 220 |
| Ser | Gln | Cys | Gly | Ala | Asn | Arg | Glu | Phe | Asp | Glu | Asn | Thr | Cys | Gln | Cys | 225 | 230 | 235 |
| Val | Cys | Lys | Arg | Thr | Cys | Pro | Arg | Asn | Gln | Pro | Leu | Asn | Pro | Gly | Lys | 240 | 245 | 250 |
| Cys | Ala | Cys | Glu | Cys | Thr | Glu | Ser | Pro | Gln | Lys | Cys | Leu | Leu | Lys | Gly | 260 | 265 | 270 |
| Lys | Lys | Phe | His | His | Gln | Thr | Cys | Ser | Cys | Tyr | Arg | Arg | Pro | Cys | Thr | 275 | 280 | 285 |
| Asn | Arg | Gln | Lys | Ala | Cys | Glu | Pro | Gly | Phe | Ser | Tyr | Ser | Glu | Glu | Val | 290 | 295 | 300 |
| Cys | Arg | Cys | Val | Pro | Ser | Tyr | Trp | Lys | Arg | Pro | Gln | Met | Ser | | | 305 | 310 | 315 |

EXHIBIT B

| | | | | | | |
|---|---|-----------------|------------|------------|---------------------------|-----|
| CCCCCCCCGC | CTCTCCAAAA | AGCTACACCG | ACGCGGACCG | CGGCGGCCTC | CTCCCTCGCC | 60 |
| CTCGCTTCAC | CTCGCGGGCT | CCGAATGCGG | GGAGCTGGGA | TGTCCGGTTT | CCTGTGAGGC | 120 |
| TTTACCTGA | CACCCGCCGC | CTTTCCCCGG | CACTGGCTGG | GAGGCGCCCC | TGCAAAAGTTG | 180 |
| GGAACGCGGA | GCCCCGGACC | CGCTCCCGCC | GCCTCCGGCT | CGCCAGGGG | GGGTGCGCCG | 240 |
| GAGGAGCCCC | GGGGAGAGGG | ACCAGGAGGG | GCCCCGCGCC | TGCGAGGGGC | GCCCCGCGCC | 300 |
| CCACCCCTGC | CCCCGCCAGC | GGACCGGTCC | CCCACCCCGG | GTCTTCCAC | C ATG CAC Met His 1 | 357 |
| TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG | Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu | 5 10 15 | | | | 405 |
| CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC | Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser | 20 25 30 | | | | 453 |
| GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT | Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala | 35 40 45 50 | | | | 501 |
| TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA | Tyr Ala Ser Lys Asp Leu Glu Glu Gln Ala Arg Ser Val Ser Val | 55 60 65 | | | | 549 |
| GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG | Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys | 70 75 80 | | | | 597 |
| TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC | Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn | 85 90 95 | | | | 645 |
| CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT | Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr | 100 105 110 | | | | 693 |
| AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA | Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln | 115 120 125 130 | | | | 741 |
| TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC | Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val | 135 140 145 | | | | 789 |
| GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT | Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys | 150 155 160 | | | | 837 |
| GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG | Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr | 165 170 175 | | | | 885 |
| AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA | Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln | 180 185 190 | | | | 933 |
| GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA | Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg | 195 200 205 210 | | | | 981 |

| | |
|---|------|
| TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225 | 1029 |
| CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr 230 235 240 | 1077 |
| TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255 | 1125 |
| CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Ser Thr Asp 260 265 270 | 1173 |
| GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290 | 1221 |
| TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305 | 1269 |
| CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320 | 1317 |
| CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335 | 1365 |
| TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350 | 1413 |
| CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370 | 1461 |
| TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385 | 1509 |
| CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400 | 1557 |
| GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415 | 1605 |
| AGC TAAGATTGTA CTGTTTCCCA GTTCATCGAT TTTCTATTAT GGAAAACCTGT Ser | 1658 |
| GTGCCCACAG TAGAAGCTGTC TGTGAACAGA GAGACCCTTG TGGGTCCATG CTAACAAAGA | 1718 |
| CAAAAGTCTG TCITTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGCTCATC | 1778 |
| TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAACAGCC AAGATTTTCC | 1838 |
| TCTTGTGATT TCTTTAAAAG AATGACTATA TAATTATTT CCACTAAAAA TATTGTTTCT | 1898 |
| GCATTCAATT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC | 1958 |
| ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT | 1997 |

EXHIBIT C

Mouse VEGF-C cDNA and deduced amino acid sequence

| | | | | | | |
|--|-----------------|------------|------------|------------|---------------------------------|-----|
| GCGGCCGCGT | CGACGCAAAA | GTTCGAGGCC | GCCGAGTCCC | GGGAGACGCT | CGCCAGGGG | 60 |
| GGTCCCCGGG | AGGAAACCAC | GGGACAGGGA | CCAGGAGAGG | ACCTCAGCCT | CACGCCCCAG | 120 |
| CCTGCGCCAG | CCAACGGACC | GGCCTCCCTG | CTCCCGGTCC | ATCCACC | ATG CAC TTG Met His Leu 1 | 176 |
| CTG TGC TTC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Leu Ile | 5 10 15 | 224 | | | | |
| CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA Pro Ser Pro Arg Glu Ala Pro Ala Thr Val Ala Ala Phe Glu Ser Gly | 20 25 30 35 | 272 | | | | |
| CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val Lys Ala Phe | 40 45 50 | 320 | | | | |
| GAA GGC AAA GAC CTG GAG GAG CAG TTG CCG TCT GTG TCC AGC GTA GAT Glu Gly Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Val Asp | 55 60 65 | 368 | | | | |
| GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys | 70 75 80 | 416 | | | | |
| CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn Thr Arg Thr | 85 90 95 | 464 | | | | |
| GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu | 100 105 110 115 | 512 | | | | |
| AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu | 120 125 130 | 560 | | | | |
| GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe | 135 140 145 | 608 | | | | |
| TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAC Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn | 150 155 160 | 656 | | | | |
| AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys | 165 170 175 | 704 | | | | |
| ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val | 180 185 190 195 | 752 | | | | |
| ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu | 200 205 210 | 800 | | | | |
| GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala | 215 220 225 | 848 | | | | |

| | |
|---|------|
| ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr 230 235 240 | 896 |
| GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG CAG GAT TTT ATC Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Gln Asp Phe Ile 245 250 255 | 944 |
| TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC Phe Tyr Ser Asn Val Gln Asp Asp Ser Thr Asn Gly Phe His Asp Val 260 265 270 275 | 992 |
| TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys 280 285 290 | 1040 |
| AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Glu Leu Asp 295 300 305 | 1088 |
| AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser 310 315 320 | 1136 |
| TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys 325 330 335 | 1184 |
| AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala 340 345 350 355 | 1232 |
| TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG Cys Glu Cys Thr Gln Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys 360 365 370 | 1280 |
| TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg 375 380 385 | 1328 |
| CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg 390 395 400 | 1376 |
| TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCATA Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn 405 410 415 | 1422 |
| CCAGTTTTC A GTCAGTCACA GTCATTTACT CTCTGAAGA CTGTTGGAAC AGCACTTAGC | 1482 |
| ACTGTCTATG CACAGAAAGA CTCTGTGGGA CCACATGGTA ACAGAGGCC C AAGTCTGTGT | 1542 |
| TTATTGAACC ATGTGGATTA CTGCGGGAGA GGA CTGGCAC TCATGTGCAA AAAAAACCTC | 1602 |
| TTCAAAGACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTT GTGATTTAAA | 1662 |
| AAAAGATGA CTATATAATT TATTTCCACT AAAAAATATG TTCTGCATT CATTTTTATA | 1722 |
| GCAATAACAA TTGGTAAAGC TCACTGTGAT CAGTATTTT ATAACATGCA AACTATGTT | 1782 |
| TAAATAAAA TGAAAATTGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT | 1836 |

EXHIBIT D

Quail VEGF-C

| | |
|---|------|
| GGCCCCGCGC AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCCCGCGG AGGGCGCGCT | 60 |
| GCGAGCGGCC ACTGGGTCCT GCTTCCCTCC TTCTCTCTCC TCCTCTCTCT CCTCCTTCTC | 120 |
| TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCGGTTT CCTGGTGGGT | 180 |
| TTTTTACCTG GCAAAGTCCG GATAACTTCG GTGAGAATTT GCAAAGAGGC TGGGAGCTCC | 240 |
| CCTGCAGGCG TCTGGGAGCT GCTGCCGCGC TCGCATCTTC TCATCCCGC GGATTTTACT | 300 |
| GCCTTGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAGAAA GGGGTGGGGG | 360 |
| GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT GCGCTGCCCC | 420 |
| GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC | 473 |
| Met His Leu Leu Glu Met Leu | 5 |
| 1. | |
| TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG | 521 |
| Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Leu Gly Pro Arg | 10 |
| 15 | 20 |
| CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG | 569 |
| Gln Pro Pro Val Ala Ala Ala Tyr Glu Ser Gly His Gly Tyr Tyr Glu | 25 |
| 30 | 35 |
| GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG | 617 |
| Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu | 40 |
| 45 | 50 |
| GAA GAG CAG TTG CGA TCT GTG TCC AGT GTG GAT GAA CTC ATG ACA GTA | 665 |
| Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val | 60 |
| 65 | 70 |
| CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA | 713 |
| Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly | 75 |
| 80 | 85 |
| GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT | 761 |
| Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp | 90 |
| 95 | 100 |
| GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA | 809 |
| Asp Ser Leu Lys Phe Ala Ala Ala His Tyr Asn Ala Glu Ile Leu Lys | 105 |
| 110 | 115 |
| AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG | 857 |
| Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val | 120 |
| 125 | 130 |
| TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT | 905 |
| Cys Val Asp Leu Gly Lys Glu Phe Gly Ala Thr Thr Asn Thr Phe Phe | 140 |
| 145 | 150 |
| AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT | 953 |
| Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Gly Cys Cys Asn Ser | 155 |
| 160 | 165 |
| GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA | 1001 |
| Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr | 170 |
| 175 | 180 |

| | |
|---|------|
| TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA | 1049 |
| Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr | |
| 185 190 195 | |
| GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT | 1097 |
| Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp | |
| 200 205 210 215 | |
| GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA | 1145 |
| Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr | |
| 220 225 230 | |
| CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC | 1193 |
| Gln Thr Gln Cys His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val | |
| 235 240 245 | |
| TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC | 1241 |
| Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe | |
| 250 255 260 | |
| TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT | 1289 |
| Ser Ser His Leu Gly Asp Ser Asp Thr Ser Glu Gly Phe His Ile Cys | |
| 265 270 275 | |
| GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA | 1337 |
| Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys | |
| 280 285 290 295 | |
| GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG | 1385 |
| Gly Gly Val Arg Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg | |
| 300 305 310 | |
| GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT | 1433 |
| Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys | |
| 315 320 325 | |
| GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA | 1481 |
| Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys | |
| 330 335 340 | |
| AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC | 1529 |
| Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys | |
| 345 350 355 | |
| GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT | 1577 |
| Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe | |
| 360 365 370 375 | |
| CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCN TGT ACA GTC CGA ACG | 1625 |
| His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr | |
| 380 385 390 | |
| AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT | 1673 |
| Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys | |
| 395 400 405 | |
| GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT | 1726 |
| Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn | |
| 410 415 | |
| CGCTATATAG TGTCG | 1741 |

EXHIBIT E

VEGF-C alignment

| | | | | | |
|-----|-------------|-------------|------------|------------|-------------|
| | 1 | | | | 50 |
| Hum | HMLLGFFSVA | CSLLAAALLP | GPREAPAAAA | AFESGLDLS | AEPDAGEATA |
| Mou | MHLLCFLSLA | CSLLAAALIP | SPREAPATVA | AFESGLGFSE | AEPDGGEVKA |
| Qua | MHLLLEMLSLG | CCLAAGAVLL | GPROPPVA.A | AYESGHGYE | EEP GAGEPKA |
| | 51 | | | | 100 |
| Hum | YASKDLEEQL | RSVSSVDELM | TVLYPEYWK | YKCQLRKGGW | QHNREQANLN |
| Mou | FEGKDLEEQL | RSVSSVDELM | SVLYPDYWK | YKCQLRKGGW | Q....QPTLN |
| Qua | HASKDLEEQL | RSVSSVDELM | TVLYPEYWK | FKCQLRKGGW | QHNREHSSSD |
| | 101 | | | | 150 |
| Hum | SRTEETIKFA | AAHYNTEILK | SIDNEWRTQ | CHPREVCIDV | GKEFGVATNT |
| Mou | TRTGDSVKFA | AAHYNTEILK | SIDNEWRTQ | CHPREVCIDV | GKEFGAATNT |
| Qua | TRSDDSLKFA | AAHYNAEILK | SIDTEWRKTQ | GMPREVCVDL | GKEFGATTNT |
| | 151 | | | | 200 |
| Hum | FFKPPCVSVY | RCGGCCNSEG | LQCHNTSTSY | LSKTLFEITV | PLSQGPKPVT |
| Mou | FFKPPCVSVY | RCGGCCNSEG | LQCHNTSTGY | LSKTLFEITV | PLSQGPKPVT |
| Qua | FFKPPCVSIY | RCGGCCNSEG | LQCHNISTNY | ISKTLFEITV | PLSHGPKPVT |
| | 201 | | | | 250 |
| Hum | ISFANHTSCR | CMSKLDVYRQ | VHSIIRSLP | ATLPQCAAN | KTCPTNYMWN |
| Mou | ISFANHTSCR | CMSKLDVYRQ | VHSIIRSLP | ATLPQCAAN | KTCPTNYVWN |
| Qua | VSFANHTSCR | CMSKLDVYRQ | VHSIIRSLP | ATQTQCHVAN | KTCPKNHVWN |
| | 251 | | | | 300 |
| Hum | NHICRCLAQE | DFMFSSDAGD | DSTDGFHDIC | GPNKELDEET | CQCVCRAGLR |
| Mou | NYMCRCLAQQ | DFIFYSNVED | DSTNGFHDVC | GPNKELDEET | CQCVCCKGGLR |
| Qua | NQICRCLAQH | DFGFSSHLGD | SDTSEGFHIC | GPNKELDEET | CQCVCCKGGVR |
| | 301 | | | | 350 |
| Hum | PASCGPHKEL | DRNSCQCVCCK | NKLFPSCGA | NREFDENTCQ | CVCKRTCPRN |
| Mou | PSSCGPHKEL | DRDSCQCVCCK | NKLFPNSCGA | NREFDENTCQ | CVCKRTCPRN |
| Qua | PISCGPHKEL | DRASCQCMCK | NKLLPSSCGP | NKEFDEEKCQ | CVCKKTCPKH |
| | 351 | | | | 400 |
| Hum | QPLNPGKAC | ECTESPQKCL | LKGKKFHHQT | CSCYRRPCTN | RQKACEPGFS |
| Mou | QPLNPGKAC | ECTENTQKCF | LKGKKFHHQT | CSCYRRPCAN | RLKHCDPGLS |
| Qua | HPLNPAKCIC | ECTESPKNCF | LKGKRFHHQT | CSCYRPPCTV | RTKRC DAGFL |
| | 401 | | 420 | | |
| Hum | YSEEVCRCPV | SYWKRPMMS* | | | |
| Mou | FSEEVCRCPV | SYWKRPHLN. | | | |
| Qua | LAEVCRCPV | TSWKRPLMN* | | | |

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

1812

DATE: 01/22/98
TIME: 15:36:47

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This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

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1
2
3 (1) General Information:
4
5 (i) APPLICANT: Alitalo, Kari
6 Joukov, Vladimir
7
8 (ii) TITLE OF INVENTION: RECEPTOR LIGAND
9
10 (iii) NUMBER OF SEQUENCES: 45
11
12 (iv) CORRESPONDENCE ADDRESS:
13 (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
14 (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
15 (C) CITY: Chicago
16 (D) STATE: Illinois
17 (E) COUNTRY: United States of America
18 (F) ZIP: 60606-6402
19
20 (v) COMPUTER READABLE FORM:
21 (A) MEDIUM TYPE: Floppy disk
22 (B) COMPUTER: IBM PC compatible
23 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
24 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
25
26 (vi) CURRENT APPLICATION DATA:
27 (A) APPLICATION NUMBER: 08/585,895
28 (B) FILING DATE: 12-JAN-1996
29 (C) CLASSIFICATION:
30
31 (vii) PRIOR APPLICATION DATA:
32 (A) APPLICATION NUMBER: US 08/510,133
33 (B) FILING DATE: 01-AUG-1995
34
35 (viii) PRIOR APPLICATION DATA:
36 (A) APPLICATION NUMBER: US 08/340,011
37 (B) FILING DATE: 14-NOV-1994
38
39 (ix) ATTORNEY/AGENT INFORMATION:
40 (A) NAME: Gass, David A.
41 (B) REGISTRATION NUMBER: 38,153
42 (C) REFERENCE/DOCKET NUMBER: 28967/33072
43
44 (x) TELECOMMUNICATION INFORMATION:
45 (A) TELEPHONE: 312/474-6300
46 (B) TELEFAX: 312/474-0448

PAGE: 2

RAW SEQUENCE LISTING
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47 (C) TELEX: 25-3856
48
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50
51 (1) SEQUENCE CHARACTERISTICS:
52 (A) LENGTH: 20 base pairs
53 (B) TYPE: nucleic acid
54 (C) STRANDEDNESS: single
55 (D) TOPOLOGY: linear
56
57 (ii) MOLECULE TYPE: DNA (genomic)
58
59 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
60
61 TGTCTCGCT GTCCTGTCT 20
62
63 (2) INFORMATION FOR SEQ ID NO:2:
64
65 (1) SEQUENCE CHARACTERISTICS:
66 (A) LENGTH: 70 base pairs
67 (B) TYPE: nucleic acid
68 (C) STRANDEDNESS: single
69 (D) TOPOLOGY: linear
70
71 (ii) MOLECULE TYPE: DNA (genomic)
72
73 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
74
75 ACATGCATGC CACCATGCAG CGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG 60
76
77 GACTCCTGGA 70
78
79 (2) INFORMATION FOR SEQ ID NO:3:
80
81 (1) SEQUENCE CHARACTERISTICS:
82 (A) LENGTH: 24 base pairs
83 (B) TYPE: nucleic acid
84 (C) STRANDEDNESS: single
85 (D) TOPOLOGY: linear
86
87 (ii) MOLECULE TYPE: DNA (genomic)
88
89 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
90
91 ACATGCATGC CCCGCCGTC ATCC 24
92
93 (2) INFORMATION FOR SEQ ID NO:4:
94
95 (1) SEQUENCE CHARACTERISTICS:
96 (A) LENGTH: 22 base pairs
97 (B) TYPE: nucleic acid
98 (C) STRANDEDNESS: single
99 (D) TOPOLOGY: linear

INPUT SET: S22772.raw

100
101 (ii) MOLECULE TYPE: DNA (genomic)
102
103 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
104
105 CGGAATTCCC CATGACCCCA AC 22
106
107 (2) INFORMATION FOR SEQ ID NO:5:
108
109 (i) SEQUENCE CHARACTERISTICS:
110 (A) LENGTH: 33 base pairs
111 (B) TYPE: nucleic acid
112 (C) STRANDEDNESS: single
113 (D) TOPOLOGY: linear
114
115 (ii) MOLECULE TYPE: DNA (genomic)
116
117 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
118
119 CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33
120
121 (2) INFORMATION FOR SEQ ID NO:6:
122
123 (i) SEQUENCE CHARACTERISTICS:
124 (A) LENGTH: 17 base pairs
125 (B) TYPE: nucleic acid
126 (C) STRANDEDNESS: single
127 (D) TOPOLOGY: linear
128
129 (ii) MOLECULE TYPE: DNA (genomic)
130
131 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
132
133 ATTTAGGTGA CACTATA 17
134
135 (2) INFORMATION FOR SEQ ID NO:7:
136
137 (i) SEQUENCE CHARACTERISTICS:
138 (A) LENGTH: 34 base pairs
139 (B) TYPE: nucleic acid
140 (C) STRANDEDNESS: single
141 (D) TOPOLOGY: linear
142
143 (ii) MOLECULE TYPE: DNA (genomic)
144
145 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
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147 CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT 34
148
149 (2) INFORMATION FOR SEQ ID NO:8:
150
151 (i) SEQUENCE CHARACTERISTICS:
152 (A) LENGTH: 40 amino acids

PAGE: 4

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

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TIME: 15:36:54

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153 (B) TYPE: amino acid
154 (C) STRANDEDNESS: single
155 (D) TOPOLOGY: linear
156
157 (ii) MOLECULE TYPE: protein
158
159 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
160
161 Pro Met Thr Pro Thr Thr Tyr Lys Gly Ser Val Asp Asn Gln Thr Asp
162 1 5 10 15
163 Ser Gly Met Val Leu Ala Ser Glu Glu Phe Glu Gln Ile Glu Ser Arg
164 20 25 30
165 His Arg Gln Glu Ser Gly Phe Arg
166 35 40
167
168
169
170 (2) INFORMATION FOR SEQ ID NO:9:
171
172 (i) SEQUENCE CHARACTERISTICS:
173 (A) LENGTH: 21 base pairs
174 (B) TYPE: nucleic acid
175 (C) STRANDEDNESS: single
176 (D) TOPOLOGY: linear
177
178 (ii) MOLECULE TYPE: DNA (genomic)
179
180 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
181
182 CTGGAGTCGA CTTGGCGGAC T
183
184 (2) INFORMATION FOR SEQ ID NO:10:
185
186 (i) SEQUENCE CHARACTERISTICS:
187 (A) LENGTH: 60 base pairs
188 (B) TYPE: nucleic acid
189 (C) STRANDEDNESS: single
190 (D) TOPOLOGY: linear
191
192 (ii) MOLECULE TYPE: DNA (genomic)
193
194 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
195
196 CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC
197
198 (2) INFORMATION FOR SEQ ID NO:11:
199
200 (i) SEQUENCE CHARACTERISTICS:
201 (A) LENGTH: 34 base pairs
202 (B) TYPE: nucleic acid
203 (C) STRANDEDNESS: single
204 (D) TOPOLOGY: linear
205

21

60

PAGE: 5

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98
TIME: 15:36:56

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207
208      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
209
210      CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC
211
212      (2) INFORMATION FOR SEQ ID NO:12:
213
214      (i) SEQUENCE CHARACTERISTICS:
215          (A) LENGTH: 20 base pairs
216          (B) TYPE: nucleic acid
217          (C) STRANDEDNESS: single
218          (D) TOPOLOGY: linear
219
220      (ii) MOLECULE TYPE: DNA (genomic)
221
222      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
223
224      GTTGCCTGTG ATGTGCACCA
225
226      (2) INFORMATION FOR SEQ ID NO:13:
227
228      (i) SEQUENCE CHARACTERISTICS:
229          (A) LENGTH: 18 amino acids
230          (B) TYPE: amino acid
231          (C) STRANDEDNESS: single
232          (D) TOPOLOGY: linear
233
234      (ii) MOLECULE TYPE: peptide
235
236      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
237
238      Xaa Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile
239      1          5          10          15
240
241      Leu Lys
242
243
244      (2) INFORMATION FOR SEQ ID NO:14:
245
246      (i) SEQUENCE CHARACTERISTICS:
247          (A) LENGTH: 17 base pairs
248          (B) TYPE: nucleic acid
249          (C) STRANDEDNESS: single
250          (D) TOPOLOGY: linear
251
252      (ii) MOLECULE TYPE: DNA (genomic)
253
254      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
255
256      GCAGARGARA CNATHAA
257
258      (2) INFORMATION FOR SEQ ID NO:15:
```

34

20

17

PAGE: 1

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION US/08/585,895A

DATE: 01/22/98
TIME: 15:36:59

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PATENT
28967/33072

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|-------------------------|---|------------------------|
| In re Application of: |) | Title: RECEPTOR LIGAND |
| Alitalo et al. |) | |
| Serial No. 08/585,895 |) | Art Unit: 1801 |
| Filed: January 12, 1996 |) | Examiner: Lathrop, B. |

Change of Inventor's Address

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:


Please be advised that the residence and mailing address of co-inventor Vladimir Joukov is now as follows:

51 Massachusetts Avenue, Apt. 1F
Boston, Massachusetts 02115

This notification is NOT intended as a change of correspondence address. Please continue to send correspondence to the Applicants' attorney at the address below:

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:


David A. Gass
Registration No. 38,153

Date: Feb 24, 1998

ASSIGNMENT

WHEREAS Helsinki University Licensing, Ltd., Viikinkaari 8 A, FIN-00710 Helsinki, Finland (hereinafter HUL), its successors and assigns, is the assignee of the entire right, title and interest in the invention or improvements of Kari Alitalo and Vladimir Joukov relating to the cloning, isolation and sequencing of human Vascular Endothelial Growth Factor C (VEGF-C) disclosed in certain applications for Letters Patent of the United States, and in said applications and any and all other applications, both United States and foreign, which Kari Alitalo and Vladimir Joukov may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension thereof; and

WHEREAS, for ten dollars (\$10.00), and other good and valuable consideration enumerated in a written agreement dated 24 October 1996, the sufficiency of which is hereby acknowledged, HUL has agreed to share ownership of the aforementioned inventions, improvements, applications, patents, reissues, extensions, and the like on a 50% / 50% equal basis with Ludwig Institute for Cancer Research, a Swiss not-for-profit corporation having an office at 1345 Avenue of the Americas, New York, New York 10105, United States of America (hereinafter LICR);

NOW, THEREFORE, HUL hereby assigns to LICR a fifty percent (50%) interest in the patent applications identified in the following LIST OF PATENT PROPERTIES, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said patent applications, and in any reissue or extension thereof.

LIST OF PATENT PROPERTIES

| <u>Application No.</u> | <u>Filing Date</u> | <u>Title</u> |
|------------------------|--------------------|---|
| 08/510,133 | 01/08/95 | Receptor Ligand |
| 08/585,895 | 12/01/96 | Receptor Ligand |
| 08/601,132 | 14/02/96 | Receptor Ligand |
| 08/671,573 | 28/06/96 | Receptor Ligand VEGF-C |
| PCT/FI96/00427 | 01/08/96 | Receptor Ligand VEGF-C |
| 08/795,430 | 02/05/97 | Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof |

WITNESS my hand this 25 day of April, Nineteen Hundred and Ninety-Seven.

Witnesses:

1) [Signature]
Name:

2) [Signature]
Name:

Helsinki University
Licensing Ltd.

By: [Signature]
Heikki Lampi
President



POWER OF ATTORNEY

The Ludwig Institute for Cancer Research hereby appoints:

Alvin D. Shulman (19,412)
Owen J. Murray (22,111)
Allen H. Gerstein (22,218)
Nate F. Scarpelli (22,320)
Edward M. O'Toole (22,477)
Michael F. Borun (25,447)
Trevor B. Joike (25,542)

Timothy J. Vezau (26,348)
Carl E. Moore, Jr. (26,487)
Richard H. Anderson (26,526)
Patrick D. Ertel (26,877)
James P. Zeller (28,491)
William E. McCracken (30,195)
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Richard A. Schnurr (30,890)
Anthony Nimmo (30,920)
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Kevin D. Hogg (31,839)
Jeffrey S. Sharp (31,879)
Martin J. Hirsch (32,237)

James J. Napoli (32,361)
Richard M. La Barge (32,254)
Karl A. Vick (33,288)
Douglass C. Hochstetler (33,710)
Cynthia L. Schaller (34,245)
Robert M. Gerstein (34,824)

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

| <u>Application No.</u> | <u>Filing Date</u> | <u>Title</u> | <u>Assignment Reel & Frame #</u> |
|------------------------|--------------------|---|--|
| 08/510,133 | 01/Aug/95 | Receptor Ligand | 8378/0566 |
| 08/585,895 | 12/Jan/96 | Receptor Ligand | 8145/0829 |
| 08/601,132 | 14/Feb/96 | Antibodies Reactive with VEGF-C, a Ligand for the Flt4 receptor Tyrosine Kinase (VEGFR-3) | 8129/0688 |
| 08/671,573 | 28/Jun/96 | Receptor Ligand VEGF-C | 8161/0909 |

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
United States of America
(312) 474-6300

Ludwig Institute for Cancer Research
1345 Avenue of the Americas
New York, New York 10105

(Date) 26-01-98

By: 

Name: A. Munro

Title: ASSOCIATE DIRECTOR



POWER OF ATTORNEY

Helsinki University Licensing, Ltd., hereby appoints:

| | | | |
|----------------------------|-------------------------------|------------------------------|---------------------------------|
| Alvin D. Shulman (19,412) | Timothy J. Vezasu (26,348) | Richard A. Schnurr (30,890) | James J. Napoli (32,361) |
| Owen J. Murray (22,111) | Carl E. Moore, Jr. (26,487) | Anthony Nimmo (30,920) | Richard M. La Barge (32,254) |
| Allen H. Gerstein (22,218) | Richard H. Anderson (26,526) | Christine A. Dudzik (31,245) | Karl A. Vick (33,288) |
| Nate F. Scarpelli (22,320) | Patrick D. Ertel (26,877) | Kevin D. Hogg (31,839) | Douglas C. Hochstetler (33,710) |
| Edward M. O'Toole (22,477) | James P. Zeller (28,491) | Jeffrey S. Sharp (31,879) | Cynthia L. Schaller (34,245) |
| Michael F. Borun (25,447) | William E. McCracken (30,195) | Martin J. Hirsch (32,237) | Robert M. Gerstein (34,824) |
| Trevor B. Joike (25,542) | David A. Gass (38,153) | | |

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

| <u>Application No.</u> | <u>Filing Date</u> | <u>Title</u> | <u>Assignment Reel & Frame #</u> |
|------------------------|--------------------|---|--------------------------------------|
| 08/510,133 | 01/Aug/95 | Receptor Ligand | 8378/0566 |
| 08/585,895 | 12/Jan/96 | Receptor Ligand | 8145/0829 |
| 08/601,132 | 14/Feb/96 | Antibodies Reactive with VEGF-C, a Ligand for the Flt4 Receptor Tyrosine Kinase (VEGFR-3) | 8129/0688 |
| 08/671,573 | 28/Jun/96 | Receptor Ligand VEGF-C | 8161/0909 |

Please continue to send correspondence to:

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(312) 474-6300

Helsinki University Licensing, Ltd.
Viikinkaari 8 A
FIN-00710 Helsinki
FINLAND

(Date)

28th of June 1998

By:

Name:

Heikki Lampi

Title:

President

Please enter the power of attorney documents into the file for the above-identified patent application.

Respectfully submitted,
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 Sears Tower
233 South Wacker Drive
Chicago, Illinois 60606-6402
(312) 474-6300

By:



David A. Gass
Registration No. 38,153

Date: Feb 24, 1998



GAU-1801
1652

PATENT #21
28967/33072
03/12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/585,895

Filed: January 12, 1996

For: RECEPTOR LIGAND

Art Unit: 1801

Examiner: Lathrop, B.

) I hereby certify that this paper is
) being deposited with the United
) States Postal Service as first class
) mail, postage prepaid, in an
) envelope addressed to: Assistant
) Commissioner for Patents,
) Washington, D.C. 20231, on this
) date:

) Dated: Feb 24, 1998

) David A. Gass
) David A. Gass

TRANSMITTAL OF POWERS OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are power of attorney documents executed by the two assignees of the above-identified patent application: Helsinki University Licensing, Ltd., and The Ludwig Institute for Cancer Research.

The above-identified application was assigned by the inventors to Helsinki University Licensing, Ltd., (HUL) in an assignment recorded at Reel 8145, Frame 0829.

HUL assigned a 50% interest in the application to The Ludwig Institute for Cancer Research, as evidenced by the attached assignment document which has been submitted for recordation.



**UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
|-----------------|-------------|----------------------|---------------------|
| 08/585,895 | 01/12/96 | ALITALO | K 28113/33072 |

HM11/0324
MARSHALL, O'TOOLE, GERSTEIN,
MURRAY & BORUN
6300 SEARS TOWER
233 SOUTH WACKER DRIVE
CHICAGO IL 60606-6402

EXAMINER

BROWN, K

ART UNIT

PAPER NUMBER

1646

DATE MAILED:

03/24/98

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.

08/585,895

Applicant(s)

Alitalo et al.

Examiner

Brown

Group Art Unit

1646

—The MAILING DATE of this communication appears on the cover sheet beneath the correspondence address—

Period for Response

A SHORTENED STATUTORY PERIOD FOR RESPONSE IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a response be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for response specified above is less than thirty (30) days, a response within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for response is specified above, such period shall, by default, expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to respond within the set or extended period for response will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Status

- ☒ Responsive to communication(s) filed on 12/1/57.
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 1 1; 453 O.G. 213.

Disposition of Claims

- ☒ Claim(s) 1, 3-5, 7, 11, 18-38 is/are pending in the application.
- Of the above claim(s) _____ is/are withdrawn from consideration.
- ☒ Claim(s) 30-36 is/are allowed.
- ☒ Claim(s) 1, 3-5, 7, 11, 18-32, 37-38 is/are rejected.
- ☐ Claim(s) _____ is/are objected to.
- ☐ Claim(s) _____ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The proposed drawing correction, filed on _____ is ☐ approved ☐ disapproved.
- ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119 (a)-(d)

- ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) _____
- ☐ received in this national stage application from the International Bureau (PCT Rule 1.7.2(a)).

*Certified copies not received: _____

Attachment(s)

- ☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____
- ☒ Notice of References Cited, PTO-892
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Interview Summary, PTO-413
- ☐ Notice of Informal Patent Application, PTO-152
- ☐ Other _____

Office Action Summary

Serial Number: 08/585,895

Page 2

Art Unit: 1646

DETAILED ACTION

1. The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1646.

Response to Amendment

2. Claims 2, 6, 8-10 and 12-17 have been cancelled, and claims 26-38 have been added.

Therefore, claims 1, 3-5, 7, 11 and 18-38 are instantly examined.

3. The following rejections are withdrawn upon reconsideration and Applicant's amendments: The rejection of claims 1 and 18-25 under 35 USC 112, first paragraph, and the rejection of claims 3-5, 11 and 18-25 under 35 USC 112, second paragraph.

4. The declaration under 37 CFR 1.132 filed 1 December 1997 is insufficient to overcome the rejection of claims 7 and 37 based upon 35 USC 112, first paragraph, as set forth in this Office action in ¶10 below for the following reasons: Although the declaration of Alitalo at ¶5 states that complete sequencing of the cDNA insert contains the sequence of SEQ ID NO:44, the declaration does not state that the cDNA insert is derived from ATCC Deposit No. 97231. The declaration also does not state what is the relationship of the 1997 base pair cDNA to the "approximately 2.1 kb insert" of the pFLT4-L clone. If the "approximately 2.1 kb insert" of ATCC Deposit No. 97231 is what was sequenced and shown to be 1997 base pairs and have the sequence of SEQ ID NO:44, then this should be made clear. In addition, Applicant must state or declare that all restrictions regarding the availability of the deposited material must be irrevocably

Serial Number: 08/585,895

Page 3

Art Unit: 1646

removed upon the granting of the patent (see Paper No. 17, page 4, and below in ¶9-11 of this Office action).

5. The declaration is also insufficient to obviate the rejection of claims 1, 18, 23-31, 37 and 38 under 35 USC 112, first paragraph, as set forth in ¶12 of this Office action for the following reasons: Although the declaration of Alitalo demonstrates at ¶7-18 that fragments comprising portions of SEQ ID NO:33 bind to the Flt4 receptor and activate tyrosine phosphorylation, and that polynucleotides which hybridize to SEQ ID NO:32 encode VEGF-C polypeptides which bind to the Flt4 receptor and activate tyrosine phosphorylation, the showing is not commensurate in scope with the claims, which recite any protein which binds to Flt4 receptor, for those reasons provided in ¶12 of this Office action.

Oath/Declaration

6. The requirement for a new declaration is withdrawn in view of Applicant's second declaration, filed 12 August 1996.

Drawings

7. The proposed drawing correction and/or the proposed substitute sheets of drawings, filed on 1 December 1997 have been approved.

8. The wish to defer formal corrections of the drawings and the petition for photographs is acknowledged.

Serial Number: 08/585,895

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Art Unit: 1646

Sequence Rules

9. The submission of a new Sequence Listing and CRF containing the sequences of Figures 9B and 10 is acknowledged, and the requirement to comply with Sequence Rules is withdrawn.

Specification

10. The amendment filed 1 December 1997 is objected to under 35 U.S.C. 132 because it introduces new matter into the disclosure. 35 U.S.C. 132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: SEQ ID NOS: 44 and 45.

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO:44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO:44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "The approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO:44." It is further noted that the nucleotide sequence of the plasmid is not SEQ ID NO:45, as stated in the added sentence. SEQ ID NO:45 is a translated open reading frame of the nucleotide sequence of SEQ ID NO:44, as stated by Dr. Kari Alitalo (§5). In addition, Applicant states that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however,

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Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the objection to the specification will be withdrawn.

Claim Rejections - 35 USC § 112

11. Claims 7 and 37 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

This rejection is maintained for reasons of record set forth in Paper No. 17, pages 4-5.

12. Applicant's arguments filed 1 December 1997 have been fully considered but they are not persuasive.

Applicant argues that ATCC Deposit No. 97231 has been deposited under the terms of the Budapest Treaty; however, Applicant must also state that all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of a patent (37 CFR 1.808), as discussed in Paper No. 17, page 4. If this statement is made, the rejection will be withdrawn.

13. Claims 1, 18, 23-31, 37 and 38 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for while being enabling for a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 sufficient to bind to the Flt4

Art Unit: 1646

receptor tyrosine kinase and stimulate tyrosine phosphorylation of the Flt4 receptor, does not reasonably provide enablement for a polynucleotide which encodes polypeptide which is defined only by its binding to the Flt4 receptor. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

Claims 1, 18, 23-31, 37 and 38 are not commensurate in scope with the specification with respect to the recitation in claims 1, 26-29 and 37 of a polynucleotide which hybridizes to SEQ ID NO:32 and which encodes a polypeptide which binds to the Flt4 receptor or in claims 18, 23-25, 30-31 and 38 of a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 which binds to the Flt4 receptor. One skilled in the art could use the guidance in the specification to isolate polynucleotides which hybridize to SEQ ID NO:32 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. Similarly, one skilled in the art could use the guidance in the specification to isolate polynucleotide which encode polypeptides which comprise portions of SEQ ID NOS:33 and test them for Flt4 receptor binding and tyrosine phosphorylation activity. However, claims 1, 18, 23-31, 37 and 38 currently recite only a polynucleotide which encodes a polypeptide which binds the Flt4 receptor, and thus these claims encompass polypeptides which bind to the Flt4 receptor under any condition and which have no biological activity. It is well-known in the art that a growth factor ligand must not only bind to its receptor but also be able to induce some biochemical signal, such as phosphorylation, in order to have a biological effect (Borg, p. 981, col. 2). Neither the specification nor the prior art teaches

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one skilled in the art how to use a polypeptide which binds to the Flt4 receptor and which does not stimulate the tyrosine phosphorylation activity of the receptor. Furthermore, given the large number of different biochemical pathways which may or may not be activated by polypeptide binding, and given the lack of guidance in the specification, one skilled in the art would not know which of the many biochemical signaling pathways, other than tyrosine phosphorylation, to examine in order to determine whether a polynucleotide which encodes a polypeptide comprising a portion of SEQ ID NO:33 might activate. Similarly, one skilled in the art would not know how to use a polynucleotide which hybridized to SEQ ID NO:32 and which encoded a polypeptide which bound to Flt4 receptor but which did not activate tyrosine phosphorylation. Absent such guidance, one skilled in the art would not know how to use a polynucleotide which encoded a polypeptide which binds to the Flt4 receptor but which does not stimulate tyrosine phosphorylation. Therefore, it would require undue experimentation to practice this invention as claimed.

This rejection could be overcome by amending the claims to recite that the encoded polypeptide not only binds to the human Flt4 receptor but stimulates tyrosine phosphorylation of the Flt4 receptor tyrosine kinase, such as is recited in claim 19.

14. Applicant's arguments regarding the previous rejection under 35 USC 112, first paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.

Art Unit: 1646

15. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

16. Claims 1, 3-5, 7, 26-29 and 37 are indefinite with respect to the term "a domain defined by eight conserved cysteine residues." It is unclear to what the eight residues are conserved. It is also unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter. Furthermore, these claims are indefinite with respect to the term "having homology to vascular endothelial growth factor." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).

17. Claims 1, 3-5, 7, 11, 18-30, 32 and 37-38 are indefinite because it is unclear what is a domain encompassed by "cysteine motifs of a Balbiani ring 3 protein." Since the BR3P domain is not defined in the specification, one cannot determine what a BR3P domain is. Furthermore, it is unclear whether the limits of the domain are defined by the cysteines, that is, the domain starts and ends with cysteine residues, or whether the domain is defined by a different parameter.

18. Claims 1, 26-29 and 37 are indefinite with respect to the term "high affinity." The term "high affinity" is relative, and it is not clear how strongly a protein must bind to the Flt4 receptor in order for it to be considered "high affinity." It is suggested that the claims be amended to recite a particular range of K_d .

Serial Number: 08/585,895

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19. Claims 1, 3-5, 7, 26-30 and 37 are indefinite with respect to the term "including." It is unclear whether "including" is equivalent to the open language "comprising" or to the closed language "consisting of."
20. Claims 3, 5, 18, 24-25 and 30-31 are indefinite because the term "said polynucleotide" lacks antecedent basis.
21. Claim 30 is indefinite with respect to the term "VEGF-homologous portion." It is not clear whether this means that the polypeptide has similarity to VEGF, or whether the polypeptide has a common evolutionary origin with VEGF (see Reeck et al. Cell, 50, 667).
22. Claim 32 is indefinite with respect to an amino acid sequence "corresponding to" another amino acid sequence. It is unclear whether "corresponding to" means that the amino acid sequence is identical or not.
23. Applicant's arguments regarding the previous rejection under 35 USC 112, second paragraph, have not been addressed as the previous rejection of the claims has been withdrawn.
24. Applicant is correct that the publication date of Reference B1 does not antedate the effective filing date of the instant application, and thus does not anticipate or render obvious the claimed invention because it is not available as prior art.
25. Applicant's arguments regarding Reference B1 are noted; however, since no rejection has been made over this patent, these arguments are not addressed.

Conclusion

26. Claims 33-36 are allowed.

Serial Number: 08/585,895

Page 10

Art Unit: 1646

27. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Brown whose telephone number is (703) 308-3667. The examiner can normally be reached on Mondays through Thursdays and on alternate Fridays from 8:30 to 6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Stephen Walsh, can be reached on (703) 308-2957.

Official papers filed by fax should be directed to (703) 305-4242. Faxed draft or informal communications with the examiner should be directed to (703) 308-0294.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

KTB

KEB

20 March 1998

Stephen Walsh
STEPHEN WALSH
SUPERVISORY PATENT EXAMINER
GROUP 1800

| Notice of References Cited | | | | Application No. 081585,895 | Applicant(s) Alitzko et al. | |
|----------------------------|---|----------------------------|---------|-------------------------------|--------------------------------|----------|
| | | | | Examiner Brown | Group Art Unit 1646 | |
| | | | | Page 1 of 1 | | |
| U.S. PATENT DOCUMENTS | | | | | | |
| * | DOCUMENT NO. | DATE | NAME | CLASS | SUBCLASS | |
| | A | | | | | |
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| | T | | | | | |
| NON-PATENT DOCUMENTS | | | | | | |
| * | DOCUMENT (Including Author, Title, Source, and Pertinent Pages) | | | | DATE | |
| | U | Reuck et al. Cell 50, 667. | | | | 1987. |
| | V | | | | | |
| | W | | | | | |
| | X | | | | | |

14. Information Disclosure Statement
3/21/97
15. Information Disclosure Statement
4/16/97
16. Office Action
5/25/97
17. Amendment and Reply
11/26/97
18. Transmittal of Powers of Attorney/Change of Inventors Address
2/24/98
19. Office Action
3/24/98
20. Amendment and Reply
7/23/98
21. Office Action
10/8/98
22. Supplemental Information Disclosure Statement
7/26/99
23. Supplemental Information Disclosure Statement
10/26/99
24. Office Action
4/4/00
25. Associate Power of Attorney
6/22/00
26. Office Action
6/29/00
27. Amendment and Reply
8/4/00
28. Amendment After Allowance/Request for Approval of Drawing Changes
1/24/01

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29. Issue Fee Transmittal
1/24/01

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